DEVELOPMENT OF HIGH YIELDING TOMATO (*Solanum lycopersicum* L.) LINES WITH RESISTANCE TO TOMATO YELLOW LEAF CURL DISEASE (TYLCD)

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN PLANT BREEDING

WEST AFRICA CENTRE FOR CROP IMPROVEMENT

COLLEGE OF BASIC AND APPLIED SCIENCES

UNIVERSITY OF GHANA

LEGON

DECEMBER, 2018

DECLARATION

I hereby declare that except for a reference to other peoples' work, which has been duly cited, this thesis is a result of my original findings and has neither in whole nor part, been presented for a degree in Ghana or elsewhere.

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is the most important vegetable in Ghana in terms of area under cultivation and consumption, but production is challenged by lack of improved cultivars and the Tomato Yellow Leaf Curl Disease (TYLCD). In Ghana, tomato breeding programmes have focused on evaluation and screening of cultivars for fruit quality and resistance to the TYLCD but very little has been done to improve the crop through breeding. The objective of this study was to introgress TYLCD resistance genes into farmer-preferred tomato cultivars. Tomato farmers were involved in the breeding programme through a Participatory Rural Appraisal in six tomato growing communities in the Ashanti, Brong Ahafo and Upper East Regions. Farmers identified TYLCD as the most important biotic stress and proposed that TYLCD resistance, high yield and long shelf life must be prioritized in tomato improvement.

To identify tomato accessions with farmers' preferred traits, diversity among 123 assembled germplasm was determined based on morphological traits valued by the fresh market and 348 SNP markers. The 123 accessions were evaluated in an augmented design with 11 accessions and two checks in each of the 11 blocks. However, 119 accessions were used for data analysis. The first five principal components explained 80% of the variation. Fruit shape, ribbing at peduncle end, fruit green shoulders, number of locules, growth type, shape at blossom end, fruits per plant, firmness, reproductive duration, yield and weight per fruit contributed to most of the variation. The accessions were grouped into two clusters with cluster I having 81 accessions and 37 accessions in cluster II. There was one outlier. A total of 338 SNP markers were polymorphic among 96 accessions. There was population overlap though major groupings were observed for PGRRI, UC Davis and improved accessions. The two most widely grown cultivars; Power Rano and Peto Mech clustered with the improved accessions from Legon, Syngenta, Wienco and Technisem.

To identify TYLCD resistant accessions, specific SCAR and SSR markers linked to the known TYLCD resistance genes were used to amplify the presence or absence of the genes in 21 tomato accessions. The accessions were thereafter screened in TYLCD hot spot in a randomized complete block design at Akumadan in the Ashanti Region and Vea in the Upper East Region. The genes ty-5 and Ty-6 were discovered in accession GH9233 (Pimplifolium) and the Ty-6 gene was found in *Pimpinellifolium* x Wosowoso. Pimplifolium expressed high level of resistance to TYLCD at both Akumadan and Vea.

To identify lines with good general combining abilities and specific crosses that show good fruit quality and yield; crosses were made between 5 locally adapted cultivars on one hand with three exotic lines and Pimplifolium each carrying two of the six TYLCD resistance genes, following North Carolina II mating design. The generated 20 F_1s were evaluated in the field in a randomized complete block design with three replications. GCA was significant for all traits studied. Peto Mech was a good combiner for fruit quality traits such as fruit length and fruit hardness. AVTO1311 x Peto Mech had positive SCA for fruit hardness. Pimplifolium x Power had the highest significant SCA estimate for fruits per plant. Lorry Tyre had the highest GCA effect for fresh tomato yield. Lorry Tyre x AVTO1311 had the highest yield per plant. Lorry x AVTO1429 had the highest fruit weight. AVTO1311 and AVTO1429 were good general combiners for fruit weight. The observed heterosis for yield and fruit quality together with the TYLCD resistance offer opportunities for the development of new hybrids. To confirm the presence of the TYLCD resistance genes, three primers genotyped 57%, 81% and 67% of *Ty-2, Ty-3 and ty-5* heterozygous alleles respectively in the F1 plants.

F_2 population from Power Rano and AVTO1429 were studied for segregation of *Ty-2* and *Ty-3* genes. Seven and three homozygous resistant plants for *Ty-2* and *Ty-3* respectively genes were identified. This will enable the screening of F_3 families in Tomato Yellow Leaf Curl Disease hotspot.

DEDICATION

I dedicate this work to my husband and children as well as my parents and siblings.

ACKNOWLEDGEMENTS

I would like to thank God Almighty for granting me the grace to successfully complete this thesis. The Director and Staff of WACCI, through whose efforts and support I got the funding from Syngenta Foundation for Sustainable Agriculture (SFSA) and German Academic Exchange Service (DAAD) for my studies.

I am also grateful to the many collaborators and practitioners, who by sharing their experiences and insights in breeding and tomato cultivation, have helped me to carry out my research successfully. These individuals and institutions include Dr. Francis K. Padi, Dr. Eugene Agbicodo, Dr. Joseph H. K. Bonney, Mr. Erasmus Kotey, Staff of Biotechnology Laboratory, Staff of the Virology Department of the Noguchi Medical Research Institute, University of Ghana, Mr. Christian Amenorkpor, Mr. Nicholas Agyekum, Mr. Yaw Kofi, Agricultural Extension Agents of Ministry of Food and Agriculture (MoFA) and the WACCI Class of 2018 (PhD Cohort 8).

Last but not least, I am grateful to my research and thesis supervision team; Prof. Samuel Kwame Offei, Prof. Kwadwo Ofori, Prof. Eric Yirenkyi Danquah and Dr. Agyemang Danquah for their guidance and reassuring faith during the period of developing my thesis

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LIST OF ABBREVIATIONS

AEA	Agricultural Extension Agents
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
AVRDC	World Vegetable Centre
BNARI	Biotechnology and Nuclear Agriculture Research Institute
CSIR	Centre for Scientific and Industrial Research
DNA	Deoxyribonucleic Acid
F_1	First Filial Generation
F_2	Second Filial Generation
FAOSTAT	Food and Agriculture Organization Statistical Database
FGD	Focal Group Discussion
FOFCREC	Forest and Horticultural Crops Research Centre
GCA	General Combining Ability
IPGRRI	International Plant Genetic Resources Institute
KNUST	Kwame Nkrumah University of Science and Technology
MAS	Marker Assisted Selection
MoFA	Ministry of Food and Agriculture
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
PRA	Participatory Rural Appraisal
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA

RELP	Restriction Fragment Length Polymorphism
SCA	Specific Combining Ability
SNP	Single Nucleotide Polymorphism
SolCAP	Solanaceae Coordinated Agricultural Project
SSR	Simple Sequence Repeats
TAS-ELISA	Triple Antibody Sandwich and Enzyme-linked Immunosorbent Assay
TGRC	Tomato Genetic Research Centre
ToMoV	Tomato Mosaic Virus
TYLCAxV	Yellow Leaf Curl Axarquia Virus
TYLCD	Tomato Yellow Leaf Curl Disease
TYLCGHV	Tomato Yellow Leaf Curl Ghana Virus
TYLCKV	Tomato Yellow Leaf Curl Kumasi Virus
TYLCMLV	Yellow Leaf Curl Mali Virus
TYLCSDV)	Tomato Yellow Leaf Curl Sudan Virus
TYLCSV	Yellow Leaf Curl Sardinia Virus
TYLCV	Tomato Yellow Leaf Curl Virus
TYLCVMaIV	Tomato Yellow Leaf Curl Malaga Virus
UC	University of California
UPOV	International Union for the Protection of New Varieties of Plants
WACCI	West Africa Centre for Crop Improvement

CHAPTER ONE

1. GENERAL INTRODUCTION

Tomato (*Solanum lycopersicum*, L.) belongs to the family Solanaceae. It is the most important vegetable in Africa but the second most important vegetable in the world after potato (FAOSTAT, 2016). Tomato originated from South America and was domesticated in Central America (Bhattarai *et al*., 2018). Tomato was introduced to Ghana between the 16th and 17th century and has since then become the most important vegetable in Ghana (Norman (1992). It also contributes significantly to the livelihood improvement of those involved in its production (Horna *et al*., 2006).

Tomato production in Africa stands at 19.79 million tonnes with an average yield of 15.59 tonnes per hectare. Comparatively, total tomato production in Ghana is approximately 366,772 tonnes with an average yield of 7.8 tonnes per hectare (FAOSTAT, 2016).

In Ghana, tomato production is seasonal and highly rain-fed (Osei *et al.*, 2013). During the rainy season, the Ashanti and Brong Ahafo Regions supply the bulk of the tomato consumed. Harvest is abundant and most of the tomatoes are wasted due to poor shelf life. As a result, prices are generally low and this serves as a disincentive to tomato farmers. The tomato produced in these areas are poor in colour, acidic and watery; making them less suitable for industrial processing (Robinson and Kolavalli, 2010).

Tomato production in the dry season does not meet the high demand. Local production is augmented by fresh tomato importation from Burkina Faso (Horna *et al*., 2006). This low production is attributed to biotic and abiotic stresses. One of the most important biotic stresses is the Tomato Yellow Leaf Curl Disease (TYLCD) caused by the whitefly-transmitted Tomato Yellow Leaf Curl Virus (Horna *et al*., 2006; Osei *et al*., 2012).

The first epidemics of TYLCD was reported in 1960 and led to total yield loss in tomato growing areas in Israel (Cohen *et al.*, 1961). The TYLCD caused severe damage in the Eastern Mediterranean Basin, North and Central Africa, Southeast East Asia, Southern Europe and Central America (Pico *et al.*, 1996). In West Africa, TYLCD has been threatening tomato production for many years and this had led to farmers in the TYLCD hotspots to misuse chemicals or abandon production during disease peak periods (Dagnoko *et al*., 2011). In Ghana, TYLCD was reported to drastically affect tomato production during the dry season and could lead to total yield loss (Osei *et al.*, 2012). A complex of fungal and viral diseases was a major problem to tomato production in Upper East Region in 2002 (Horna *et al.*, 2006). In 2014, farmers in the Agotime-Ziope District of the Volta region were reported to have lost virtually all their investment following the TYLCD infection of over 1,000 hectares of tomato farms in the area (Duodo, 2014).

Over the years, cultural, chemical and physical approaches have been used to control TYLCD, but with limited success. The best strategy to combat TYLCD is to breed for Tomato Yellow Leaf Curl Disease resistance. The wild tomato relatives served as source of TYLCD resistance since no resistance gene was identified *Solamum lycopersicum* (Pico *et al*., 1996). Breeding for resistance involves either introducing the disease resistance genes from wild tomato species into cultivated tomato (Pilowsky and Cohen, 1990) or the transgene strategy; introducing viral genes into cultivated tomato (Kunik *et al*. 1994). The past three and half decades have been devoted to development of TYLCD-resistant cultivars through the identification of resistant sources, generation of single gene resistant breeding lines and identification of molecular markers linked to resistance genes (Gordon, 2009). Six TYLCD resistance genes *Ty1/3*, *Ty-1*, *Ty-2*, *Ty-4 ty-5* and *Ty-6* have been identified and currently, the focus is on pyramiding of multiple TYLCD resistance genes into single cultivars (Hanson *et al*., 2016).

In Ghana, there has been little effort devoted to the development of improved tomato varieties. Past breeding programmes focused on evaluation and screening of exotic and local cultivars for fruit quality (Blay *et al*., 1999; Gongolee 2014) and TYLCD resistance. Most of the exotic cultivars have better fruit quality but are susceptible to TYLCD. The local cultivars, *Pimpinellifolium* and its derivatives tend to be tolerant to the TYLCD at various locations (Osei *et al*., 2013; Asare-Bediako *et al*., 2017 and Segbefia *et al*., 2018). However, crosses between *Pimpinellifolium* and Roma, Wosowoso and Chery tomato resulted in fruits that were tiny and unsuitable for the fresh market (Segbefia *et al*., 2015).

To lessen the impact of TYLCD on tomato production in Ghana, it will be necessary to identify which of the TYLCD resistance genes in elite breeding lines will be effective against the local strains of TYLCV in Ghana. This could be followed by Marker Assisted Selection (MAS) of lines that combine both fruit quality with TYLCD resistance genes.

The development of tomato lines with high fruit yield and desirable fruit quality for fresh market as well as tolerant to TYLCD were major considerations in this study. In this regard, the main research objective was to introgress TYLCD resistance genes into farmer-preferred tomato cultivars in Ghana.

The specific objectives of the study were to:

1. identify production constraints and farmers' preferred traits of tomato cultivars;
2. identify accessions with superior fruit and yield-related traits;
3. determine genetic diversity of tomato germplasm in Ghana;
4. identify local and exotic tomato germplasm with TYLCD resistance to Ghanaian isolates of Tomato Yellow Leaf Curl Virus (TYLCV); and
5. identify F_2 plants with homozygous TYLCD resistance gene loci.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of tomato

2.1.1 Origin and domestication of tomato

The wild relatives of cultivated tomato (*Solanum lycopersicum* L.) originated from Western South America from northern Ecuador, through Peru, to northern Chile and in Galapagos Island (Peralta and Spooner, 2007). According to Rick and Holle (1990), the closest ancestor of cultivated tomato is the wild cherry tomato (*S. lycopersicum var. cerasiforme*).

Over the years, wild tomatoes have been domesticated through intensive selection and plant breeding. Two hypotheses have been propounded for the original site of tomato domestication; one stipulates Peru (de Candolle 1882) and the other Mexico. It is, however, presumed that Mexico is probably the site of domestication and Peru is the centre of diversity (Larry *et al.*, 2007). The first hypothesis was based on botanical evidence complemented with linguistic, historical aspects (Luckwill, 1943) and recently molecular studies (Nesbitt and Tanksley, 2002). The second hypothesis is supported by the evidence of pre-Columbian cultivation in Mexico but no evidence in South America. This is further supported by the argument that the name tomato comes most probably from the Mexican Nahua peoples' word 'Tomatl' which describes 'plants bearing globous and juicy fruit (Sahagún, 1988). The biloculed domesticated forms found in south Mexico and Guatemala are the oldest cultivated types (Harlan, 1971).

Tomato is not an indigenous crop in West Africa; it was introduced to Africa in the 16th century by European merchants and has since been integrated into various dishes. Tomato cultivation in West Africa was documented in 1898 and by 1920s, tomato was sold in local markets (Tew, 1920). Norman (1992) asserts that tomato was introduced to Ghana between the 16th and 20th century and has since become an important component of most Ghanaian meal.

2.1.2 Taxonomy of tomato

Galen, a Greek naturalist in the 14th century named a plant that was unknown as *Lycopersicon*, which means ‘wolf peach’. When tomato was first introduced into Europe in the 16th century, early European botanist recognized the close relationship of tomato with the genus *Solanum* and commonly referred to them as *S. pomiferum* (Luckwill, 1943). However, Anguillara (1561) identified the newly introduced tomato as the plant named *Lycopersicon* by Galan, hence Tournefort (1694) considered cultivated tomato to be within the distinct genus *Lycopersicon* and used the multilocular nature of the tomato fruit to distinguish *Lycopersicon* from *Solanum*. In the following century, Linnaeus (1753) classified tomato into the genus *Solanum* and under the specific name *Solanum lycopersicum*. Miller (1754) agreed with Tournefort’s classification and officially described the genus *Lycopersicon*. Jessieu (1789) included tomato in the genus *Solanum*. In addition, Linnaeus described a second wild species from Peru, *S. peruvianum* (Jessieu, 1789). The classification of tomato under *Lycperisicon* continues until the 1990s (Rick *et al*., 1990).

With the advent of molecular tools, the phylogenetic relationships within the Solanaceae were examined and Spooner *et al*. (1993) examined the outgroup relationship of tomato and other members of the Solanaceae based on chloroplast DNA restriction site data. Further molecular studies confirmed that tomato is in the genus *Solanum lycopersicum* (Peralta and Spooner 2001). Taken into consideration the morphological characters, phylogenetic relationships and geographical distribution, thirteen species of wild tomato including the cultivated tomato (*Solanum lycopersicum* L.) and four closely related species have been grouped as part of the tomato clade.

2.2 Climatic and soil conditions for tomato production

The countries with higher yields do not have ideal conditions for tomato production, neither do they devote more land for tomato production. These countries largely produce tomato under greenhouse conditions (Peralta and Spooner, 2001).

Tomato requires a minimum of 8 hours of continuous sunlight per day, 3 to 4 months of warm, clear and fairly dry weather to produce the best of fruits. The optimum temperature ranges for tomato growth range from 21 to 27 ^{o}C (Van Dam, 2005). The night temperature requirement of tomato falls between 12.8 ^{o}C and 23 ^{o}C to set fruits. Beyond night temperatures of 29 ^{o}C there will be poor color development in tomato. High temperature above 32 ^{o}C adversely affect flower formation, fruit setting, vegetative growth development and yield (Berry and Rafique-Ud-Din, 1988). Growth totally ceases when temperatures are over 34.7 ^{o}C throughout the day.

Tomato requires loose, well-drained soil but will grow in any garden soil. The ideal soil type for early tomato variety is sandy loam while clay loam is suitable for late varieties. The optimum soil pH for tomato growth is between 6.0 and 7.0. The soil should be evenly moist with regular supply of water and maintained at about 60% field capacity (Tran, 2005).

2.3 Economic importance of tomato

Tomato is the second most important vegetable in the world based on area under cultivation as well as income generation (FAOSTAT, 2016). Tomato is consumed raw or processed into various forms such as paste, puree, juices and whole peeled tomato. It is an important source of various nutrients. Fresh tomato is composed of vitamin A and C, minerals, high amount of water and low calories. The stage of maturity of tomato fruits affect the vitamin A content of the fruit. Tomato is also an important source of carotenoids (Wilcox *et al*., 2003) and lycopene; a natural antioxidant (Shih *et al*., 2004).

The five leading producers of tomato in the world are China, India, United States of America, Turkey and Egypt. The world tomato production in 2014 was 177 million tonnes with average yield of 37 tonnes per hectare (FAOSTAT, 2016).

Tomato is the most important vegetable in Ghana (Osei *et al*., 2008). It is an indispensable nutrient in most Ghanaian dishes (Tambo and Gbemu, 2010). Tomato production in Ghana presented a huge opportunity for economic growth and this has led to increased production as well as the establishment of tomato processing factories across the country. By 1968, three stated-owned tomato factories were established in Ghana. These three tomato processing factories were located in Pwalugu (Northern belt), Wenchi (middle belt) and Nsawam (Southern belt). The highest average yield recorded in Ghana's history was 10.2 t/ha in 1996 and 1998 while the lowest recorded yield was 3.8 t/ha in 2005. As of 2016, the average tomato yield was about 7.8 t/ha and production was 366,772 tonnes (FAOSTAT, 2016). Tomato production in the country was further enhanced by the completion of the Tono dam in 1985 which served an area of 24,000 hectares for growing crops.

2.4 Constraints to tomato production

Tomato production is faced with many challenges throughout the world. The constraints encompass adaptability, fruit quality, biotic and abiotic stresses. In Ghana, tomato cultivars mostly grown have poor colour, poor shelf life and watery (Robinson and Kolavalli, 2010). Abiotic constraints include inadequate irrigation facilities and high temperature (Horna *et* al., 2008). Among the biotic constraints of tomato production, pests and diseases are the most devastating. Throughout the world, the three most important diseases of tomato are Tomato Yellow Leaf Curl Virus Disease (TYLCD) caused by Begomoviruses, late blight caused by *Phytopthora infestans* and bacterial wilt caused by *Rastonia solanacearum*. These diseases are very important because the pathogens responsible for them are genetically diverse and frequent mutation gives rise to new forms. Various resistance genes have been identified and introgressed into commercial cultivars (Hanson *et al.*, 2016). Among these three most important diseases, TYLCD is the most devastating tomato disease in Ghana. Breeding to address these major constraints in tomato production usually commences with characterization of existing germplasm (Ziaf *et al*, 2016) for adaptability, fruit quality or disease resistance.

2.5 Diversity in tomato

Tomato is a diploid (2n=24), however, Rick (1976) reported some spontaneous tetraploids. The genetics of tomato has been extensively studied over a century ago and this has resulted in a deeper understanding of the genetics, breeding and evolution. Originally, tomatoes were pea-sized berries but domestication and plant breeding have resulted in increased fruit sizes (Soyk *et al.*, 2017). The genetic variation within cultivated tomato is about 5% (Miller and Tanksley 1990). Nonetheless, there has been constant interspecific hybridization in tomato breeding and this has resulted in higher genetic distance and greater allelic richness in contemporary cultivated varieties compared to landraces and vintage varieties. In addition, breeding for market specialization and fruit characteristics has resulted in genetic differentiation within contemporary varieties (Sim *et al.*, 2011). Initially, introgressing agronomic traits from the wild into cultivated tomato had challenges such as linkage between favourable and unfavourable effects of introgressed fragments. However, with the advent of molecular biology in the 80's, significant progress has been made in characterizing the genetic diversity in both wild and cultivated tomato as well as intraspecific and interspecific hybridization.

2.6 Estimation of genetic diversity in tomato

Through domestication and artificial selection, the genomic compositions and population structure of available germplasm resources have changed (Huang *et al*. 2012). This variability has been standardized in the International Plant Genetic Resources Institute (IPGRI, 1996) tomato descriptor and the International Union for Protection of New Varieties of Plants (UPOV, 2011) tomato descriptor. The classification and standardized evaluation take into consideration several morphological attributes such as fruit weight, fruit shape and colour (Paran and Van Der Knaap, 2007), physico-chemical, sensory quality and nutritional composition.

In-depth analysis of population structure is a prerequisite for any breeding programme in order to get targeted improvement in the traits. Understanding genetic variation is important for genetic resource conservation and breeding programmes. Estimation of diversity in germplasm can be carried out in various ways such as evaluation of phenotypic variation or morphological characterization, biochemical characterization and molecular characterization.

2.6.1 Morphological characterization of tomato

Morphological characterization is a simple technique of quantifying genetic variability. Nonetheless, both morphological and biochemical characterizations are not much reliable due to genotype by environment interaction.

Availability of diverse germplasm, as well as characterization of diverse germplasm, is a prerequisite for any breeding programme (Ziaf *et al.*, 2016). Cultivated tomato has a very limited genetic variability compared to its wide relative (Miller and Tanksley, 1990) and this makes it difficult to identify polymorphisms between elite germplasm (Sim *et al*., 2009). It has also been reported that the diversity of cultivated tomato has been reduced over the past decades due to the disappearance of local varieties (Yi *et al*., 2008) and the breeding for market specializations as well as geographic adaptations (Sim *et al*., 2011). Initially, genetic variation studies focused on the differences between the wild species and cultivated varieties. Recent studies have focused on the variations within modern varieties (Corrado *et al*., 2013).

Genetic variation in wild species has been a source of fruit quality trait, disease and insects resistance in modern plant breeding programmes (Rick and Chetelat, 1995). Understanding the genetic diversity is very significant in plant breeding applications (Mohammadi and Prasanna, 2003) such as analysis of genetic variability in cultivars (Smith, 1988), identification of parental combinations needed for generating segregating populations with maximum genetic variability for further selection (Barrett and Kidwell, 1998) and introgression of desirable genes from diverse germplasm into available genetic base (Thompson and Nelson, 1998). To develop desired tomato cultivars, it is necessary to catalogue genetic variability within the available germplasm (Islam *et al*., 2004).

2.6.2 Molecular characterization of tomato

Molecular characterization is preferred due the ubiquitousness, repeatability, stability and the high reliability of this method. Molecular characterization is not affected by the environment and exhibits higher levels of polymorphism. Over the years, molecular markers developed and used for characterization and variety identification included Restriction Fragment Length Polymorphism (RELP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs).

Molecular techniques have been utilized to pinpoint genomic regions of specific traits. Genetic control of complex traits has been understood using ad hoc techniques which have led to the identification of key alleles controlling diverse agronomic traits originating from the wild. Presently, the knowledge of the diversity in the tomato has been enhanced with the full sequence of the tomato genome, the emergence of omics and next generation sequencing techniques. Employing QTL mapping technique in natural populations or genome-wide association studies facilitates the genetic characterization of complex traits and germplasm management in both wild and cultivated tomato. Several breeders have studied genetic diversity in tomato germplasm for improvement of various growth and yield-related traits (Blay *et al*., 1999; Saleem *et al*., 2015).

2.7 Improving tomato for yield and fruit quality traits

Over the years, tomato breeding activities have focused on improving fruit quality and or breeding for stress tolerance. Understanding the genetic and molecular diversity for fruit quality, yield and yield component parameters are important for selecting diverse parents. Most fruit quality characteristics that have been researched include size, shape, total solids or brix, pH, colour, firmness, ripening, nutritional quality and flavor. Fruit colour is of much importance to the fresh market and this is on the rise because of the increasing awareness of the health benefits of carotenoids. Total solids have been extensively worked on because of its importance to the processing industry. In general, fruit shape is a quantitatively inherited trait and it involves 4 to 17 loci (Gonzalo and van der Knaap, 2008). However, elongated fruit shape and blockiness in tomato is controlled by only one major locus (Grandillo *et al*., 1996). Elongated fruit shape and blockiness are preferred for processing (van der Knaap and Tanksley, 2003) since these traits prevent the fruit from rolling off the conveyer belts (Visa *et al*., 2014). Blockiness can be at stem-end or blossom-end. Blossom-end blockiness was strongly correlated with fruit size (van der Knaap and Tanksley, 2003). Stem-end blockiness was reported to be controlled by the same loci that controlled heart shape and both are strongly correlated (van der Knaap and Tanksley, 2003). Cultivated tomatoes vary in sizes which range from 5 mm in tom berries and 1-2cm in cherry tomato to 10 cm or more in diameter (beef steak). Due to market specialization commercial varieties are typically 5-6 cm in diameter (Patil, 2015). Fruit, shape and size are affected pleiotropically by locule number (Rodriguez *et al*., 2011). The shelf life of tomato is very important for transportation, marketing and domestic use. Shelf life is controlled by both genetic and environmental factors particularly temperature.

Besides biochemical changes, fruit firmness, number of locules per fruit, fruit pericarp thickness influence the shelf life of tomato (Bekov, 1968).

Improvement of tomato fruit quality in Ghana commenced in the 1950s when cultivars such as OK, MH series (Mc Ewen, 1961) and Wosowoso (Schippers, 2000) were developed. Agble (1978) began breeding for processing quality traits, shelf life and heat tolerance by making crosses between local accessions with heat tolerance and exotic accessions with nonripening gene (nor^{A}). Nevertheless, due to lack of continuity, no variety was released despite the positive outlook (Orchard and Suglo, 1999).

In addition, between 1994 and 2000, a research team led by the National Research Institute (NRI) in the UK focused on pure line selection of local landraces in the Brong Ahafo Region of Ghana with the ultimate aim of releasing pure strains of good open-pollinated varieties. Six varieties consisting of three local and three introduced varieties were used in that study. These varieties were selected based on farmers and traders preferred traits (fruit quality, good taste and longer shelf life). A tomato breeder seed production trial was then established at Wa in the Upper West Region with five selected varieties. The research was not very successful because there was no long-term impact due to lack of sustainable seed distribution systems to ensure that the resource-poor farmers have access to the developed varieties. Tomato varieties that are currently grown by Ghanaian farmers are mostly imported varieties and farmer-selected varieties. A very important pure line grown in Ghana particularly in the Brong Ahafo Region is the Power Rano (a cross between Power and Laurano varieties) which was identified by the NRI researchers in the 1990s based on its good production and local processing qualities (Robinson and Kolavalli, 2010).

However, since the NRI tomato breeding work ended in 2000 there have been no breeding programmes and no systematic seed multiplication in the country (Robinson and Kolavalli, 2010; Osei *et al*., 2013).

2.8 Tomato Yellow Leaf Curl Disease (TYLCD)

2.8.1 History of TYLCD

One important stress of tomato that has been focused on extensively is the TYLCD. TYLCD has been reported to be the most devastating tomato disease in the tropics and subtropics. It affects tomato grown in open fields or greenhouse and caused yield loss up to 100% in many countries including Ghana (Polston and Anderson, 1997; Horna *et al*., 2006; Yongping *et al*., 2008).

It was first reported in the Middle East in 1931 but has since then spread to various parts of the world (Czosnek and Laterrot, 1997). In the 1960s the first epidemic of TYLCD was reported in Israel (Urbino *et al*., 2008). This was followed by a report in Europe (Kheyr-Pour *et al*., 1991), Asia (Rochester, *et al*., 1990), South America, Africa (Harrison *et al*., 1991) and Australia (Dry *et al*., 1993). TYLCD originated from the Middle East and the symptom was first reported in the Jordan Valley in Israel in the late 1920s (Pico *et al*., 1996). At the time of discovery of this disease, the plant growth was characterized by severe stunting, erect shoots, small and yellowing of leaves. As the disease progressed, the leaves became chlorotic and curled upward. Plants that were affected at an early stage produced no marketable fruits. The name Tomato Yellow Leaf Curl Disease (TYLCD) was coined based on the symptom expression (Cohen and Harpaz, 1964). By the summer of 1959, there was a serious outbreak of the disease that led to total yield loss in tomato growing regions in Israel (Cohen *et al*., 1961) and this devastating loss was strongly correlated with significant increase in whitefly (*Bemisia* *tabaci*) population. Again, it was noticed that the population of whitefly skyrocketed due to the initiation of large-scale cotton cultivation in Jordan and Bet She'an Valleys. The cotton served as an alternate host for the white fly (Cohen and Lapidot, 2007). Further laboratory-controlled transmission tests confirmed the disease was whitefly vectored and viral in nature (Cohen and Harpaz, 1964). Since the causal agent is a virus it was named as Tomato Yellow Leaf Curl Virus (TYLCV). By the 1965 TYLCD symptoms were described in some African countries such as Sudan and Egypt in 1966 (Makkouk and Laterrot, 1983). By the mid-1970s similar virus symptoms were reported in West African countries like Nigeria (Lana and Wilson, 1976) and by late 1970s and early 1980s it had spread to Senegal, Cape Verde, Gambia, Mauritania, Cote d'Ivoire and Mali (D'hondt and Russo. 1985). The symptoms observed in all these countries were not the same but similar. Molecular tools were used to characterize the virus in the late 1980s (Navot *et al*., 1989). It was realized that the different clades of virus species have diverged from Mediterranean and African viral species as far back as 130 million years ago with the separation of Americas from Gondwana landmass (Seal *et al*., 2006).

2.8.2 Tomato Yellow Leaf Curl Virus (TYLCV)

TYLCV is a virus from the genus Begomovirus and the family Geminiviridae. Begomoviruses are the largest and most economically important genus of the family Geminiviridae (Fauquet and Stanley, 2005). The viruses have a wide host range including tomato, tobacco, pepper, petunia, melon, watermelon, squash, gourd, common bean, soybean, lima bean, mung bean, cowpea, cassava, cotton and okra (Seal *et al*., 2006). Geminiviruses are characterized by unique Gemini shape of fused icosahedral viral particle and the genus consist of viruses with both monopartite and bipartite genomes. The TYLCV has a single-stranded circular DNA and a genome size of about 2.8 kb (Moriones and Navas-castillo, 2000).

2.8.3 Phylogeny of TYLCV

Phylogenetic studies have determined that TYLCD associated viruses from the Mediterranean Basin and northern Sub –Saharan Africa consist of six distinct virus species commonly referred to as TYLCV cluster (Abhray *et al*., 2007). The first TYLCV cluster is Tomato Yellow Leaf Curl (TYLCV) – a Mediterranean species first isolated in Israel, the second cluster is Tomato Yellow Leaf Curl Sardinia Virus (TYLCSV), a Western Mediterranean species common in Italy and Spain. Recombination between TYLCV and TYLCSV has resulted in two viral species, Tomato Yellow Leaf Curl Malaga Virus (TYLCVMaIV, Monci *et al*., 2002) and Tomato Yellow Leaf Curl Axarquia Virus (TYLCAxV, Garcia-Andres *et al*., 2006). The third, fourth, and fifth clusters were found in West Africa; Tomato Yellow Leaf Curl Mali Virus (TYLCMLV) present in Mali (Dembele and Noussourou, 1991), Ghana (Osei *et al*., 2008), Benin, Burkina Faso, Niger, Senegal and Togo (Chen *et al*., 2009) as well as Ethiopia (Shih *et al*., 2006). The sixth cluster is the Tomato Yellow Leaf Curl Sudan Virus (TYLCSDV) found in Sudan and Yemen (Abhary *et al*., 2007).

In addition to the six TYLCV clusters, there are 51 whitefly transmitted tomato virus species (Fauquet and Stanley, 2005).

2.9 Mode of Acquisition and transmission of TYLCV

The TYLCV is mostly transmitted by the whitefly, *Bemisia tabacci* biotype B (Gennadius), which also belongs to the family Aleyrodidae, and order Hemiptera. Whitefly is also known as one of the most important and widely distributed insect pests in the tropics and subtropics (Brown *et al*., 1995).

Whitefly ranges between 1 to 2 mm in size and is normally found in clusters on the underside of the leaves. They are very active during the daytime and are capable of reproducing throughout the year in warm climates. The larva and adult whiteflies pick up virus particles when feeding on infected plants. It takes 16-35 days for the whitefly to develop from egg to adult depending on the temperature.

With the continuous movement of the adult whitefly and subsequent feeding on susceptible healthy plants, the virus is spread from one plant to the other. The whiteflies feed by sucking plant juices from the phloem and cause injury to the plant in several ways. Due to the high population of whiteflies feeding at the same time, there are normally chlorotic spots at the feeding sites on leaf surfaces and the production of a sticky substance known as honeydew. The honeydew produced may lead to discoloration of leaves and fungi diseases on the surface of the leaves when conditions are favourable.

Transmission of the virus by the whitefly is in a persistent circular non-propagative manner. There has been evidence that TYLCV can be transmitted from infectious males to female or vice versa through copulation (but not among insects of the same sex) and subsequently transmitted to a tomato plant. It can also be transovarially transmitted to progeny through the egg for two generations (Ghanim *et al*., 1998). This insect to insect transmission can increase the population of whiteflies that are able to transmit the virus.

TYLCV is poorly transmitted by mechanical inoculation and grafting. TYLCV cannot be transmitted by contact between plants. Recently, Kil *et al* (2016) presented the first report of TYLCV seed transmission in tomato plants.

Tomatoes serve as the primary host for the vector. However, there are other alternate hosts like cucumbers, cotton, eggplants, potatoes, tobacco, beans, peppers and some weeds among over 300 species within 63 families (Mound and Halsey, 1978).

2.10 Breeding for TYLCD resistant varieties

Worldwide breeding for TYLCD resistance varieties began in the 1960s and has been based on the identification of resistance genes from wild species of tomato and followed by introgression of TYLCD resistance genes into cultivated tomato. This is because cultivated tomato is inherently susceptible to the viruses (Vidavsky and Czosnek, 1998). However, the success has been slow due to the complex genetics of the resistance (Lapidot and Friedmann, 2002) and the numerous virus strains as well as differences in virulence. Breeding for resistance can be achieved by making crosses between wild tomato and cultivated tomato, followed by phenotypic or molecular selection of progenies from segregating populations or from backcrosses (Barbieri *et al*., 2010). Progress in developing resistant varieties with the conventional approach was slow due to the type of resistance and the different strains of the virus causing TYLCD (El-Dougdoug *et al*., 2013). However, there has been considerable progress with the dawn of marker-assisted breeding. The first commercially tolerant cultivar was TY-20, with resistance from *L. peruvianum.* This cultivar was known to show delayed symptoms and accumulation of viral DNA (Rom *et al*., 1993).

2.10.1 Identification of resistant genes

Depending on the plant source, resistance has been reported to be controlled by one to six genes and these genes have been introgressed into cultivated tomato (Ji *et al*. 2009 and Hanson *et al*., 2016). Breeding for TYLCD resistance started with the use of conventional breeding techniques. This involved the identification of a source of resistance, usually from the wild tomato species. These sources of resistance include *S. pimpinellifollium, S. peruvianum, S. chilense, S. habrochaites* and *S. cheesmaniae* (Ji *et al*., 2007a).

2.10.2 Sources and mapping of TYLCD resistant genes

Most of the TYLCD resistance genes with the exception of *Ty-2* and *Ty-5* were all derived from *S. chilense*. *Ty-1*, *Ty-3* and *Ty-3*a genes were derived from *S. chilense* accessions LA1969, LA2779 and LA1932 (Scott *et al*., 1996; Ji *et al*., 2007a) respectively. All three (3) genes were located on the long arm of chromosome 6 (Zamir *et al*., 1994). *Ty-3* is 15 cM away from *Ty-1* and both genes have been demonstrated to be allelic (Verlaan *et al*., 2013). LA1932 was the donor for *Ty-4* but mapped to chromosome 3 (Ji *et al*. 2009). Recently, *Ty-6* was derived from LA2779 (also a donor for *Ty-3*) and mapped to chromosome 10 (Hutton *et al*., 2012).

Ty-2 was derived from *S. habrochaites f. glabratum* accession B6013 and was mapped to chromosome 11 (Hanson *et al*., 2000, Yang *et al*., 2014) while *ty-5* was mapped to chromosome 4 (Anbinder *et al*., 2009) and first derived from a breeding line TY-172; a progeny purported to be developed from a cross of four *S. peruvianum* accessions. Nonetheless, there is an ongoing debate as to whether *ty-5* originated from *S. peruvianum*. Recently, it emerged that *ty-5* is recessively inherited (hence the symbol *ty-5*).

2.10.3 Effectiveness of TYLCD resistance genes against TYLCD

Ty-1 and *Ty-2* genes express complete or nearly complete dominance (Hanson *et al*., 2000; Zamir *et al*., 1994) and effective against only monopartite TYLCV whereas *Ty-3* is incomplete dominant or more additive (Ji *et al*., 2007a) and effective against both TYLCV and the bipartite ToMoV (Agrama and Scott, 2006; Ji *et al*., 2007a). *Ty-4* alone is also effective against TYLCV, however, combining *Ty-3* and *Ty-4* had greater resistance than *Ty-3* alone in Guatemala (Ji *et al*., 2009). The resistance of genotypes homozygous for *Ty-3* and *Ty-4* was significantly higher than genotypes with *Ty-3* alone, which in turn were significantly more resistant than commercial hybrids heterozygous for *Ty-1* (Ji *et al*., 2009). *Ty-6* has an additive effect and is known to be highly effective with *Ty-3* or *Ty-5* and it is broadly effective against monopartite and bipartite virus. *Ty-6* has moderate resistance to TYLCV but high resistance to ToMoV (Scott and Hutton, 2015).

Ty-1 and Ty-2 are both dominant and provide high levels of resistance to many strains of TYLCV, they are widely utilized by breeders. Yet, neither gene is effective against bipartite begomoviruses, and the resistance of both genes has been overcome by some strains of TYLCV

(Ji *et al*., 2007a). There is evidence, however, that *Ty-2* can provide an enhanced level of resistance to bipartite begomoviruses when pyramided with *Ty-3*, potentially making it a more attractive tool to breeders. In 2009, scientists at the World Vegetable Center (AVRDC) pyramided multiple TYLCD resistance genes into their breeding lines. Various breeders have used these breeding lines to develop tomato with resistance against the TYLCD (DFID research, 2012). In addition, AVRDC gives high priority to the incorporation of *Ty-3* into new breeding lines. Their current results from TYLCD screening indicate that *Ty-3* reduced tomato Yellow Leaf Curl Disease symptom severity but did not prevent virus infection. Similarly, they found out that *Ty-3* and *Ty-2* combination did not eliminate the virus and recommended that pyramiding new resistance gene combinations such as *Ty-3*, *ty-5* and *Ty-2* should be explored (Hanson *et al*., 2016).

2.10.4 Populations for marker assisted breeding of TYLCD resistant varieties

Known primers for the various TYLCD resistance genes can be used to screen for polymorphism in breeding population to identify lines or plants that carry the resistant genes. Field screening in a hot spot or controlled inoculation can also be carried out to identify lines or plants that are resistant to TYLCD.

Primers can be used to determine heterozygous or homozygous loci of a particular Ty gene in early generation such as F_2 progenies and F_3 families derived from marker selected F_2 plants (Barbieri *et al*., 2010). This can be supplemented by challenging these populations with different isolates of the TYLCV either under controlled environment or in a disease hot spot.

In pyramiding TYLCD resistance genes, two F_2 populations (from a biparental cross) segregating for *Ty-3* and *Ty-2* were used as a base population and then further advanced to F_6 based on disease response and horticultural traits (Prasanna *et al*., 2015). A three-parent cross was used to develop multiple disease resistant lines. TYLCD intensive selection was carried out at F_1 - F_4 generations. Plants identified with MAS were further assessed in the field for Tomato Yellow Leaf Curl Disease severity (Hanson *et al*., 2016).

2.10.5 Methods of screening lines against TYLCD

The deployment of phenotypic and molecular selection procedures leads to cost-effective and speedy development of TYLCD resistance. An efficient phenotypic screening procedure validates the marker-assisted selection.

Various screening procedures for TYLCD resistance have been used over the years; however, the choice of screening method would depend on the reliability and cost-effectiveness considering the population size. Generally, there are two screening methods namely field screening in a disease hotspot and greenhouse or growth room screening method (Controlled Environment).

Field screening methods should be considered when breeding is done in the region where the cultivar will be released and there are established disease hotspots. Greenhouse screening or growth room screening permits the screening of materials irrespective of the region where it will be released. It only requires the right source of inoculum for the screening process and the mimicking of the environmental conditions that will exist in the target region. Greenhouse screening enables quick assessing of disease reaction, escape from other pest and diseases, reduces sources of environmental variation by the use of the particular pathogen strain and the appropriate pathogen concentration (Hanson *et al*., 2016).

2.10.5.1 Disease severity rating of TYLCD

In order to group genotypes on the basis of their level of resistance to TYLCD, several scoring scales have been used for TYLCD disease severity. One of the most commonly used is the scale developed by Lapidot and Friedman (2002). Severity scores are based on 0 = 4 scale where; 0 = No symptoms; 1 = Slight yellowing (mild symptom); 2 = Leaf curling and yellowing (moderate symptom); 3 = Yellowing, Curling and Cupping (severe symptom); 4 = Severe stunting, curling and cupping; plant stops growth (very severe symptom). Using this scale, scoring of plants is done at 30, 60 and 75 days after transplanting.

Friedmann *et al*. (1998) also used the following scale; 0 = no visible symptoms, inoculated plants show the same growth and development as non-inoculated plants; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop; and 4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stops.

Another scale, though different from the first two discussed above, ranges from 0-3 as follows; 0 = symptomless and 3 = severe symptoms. All plants that scored 0 were classified as resistant and all plants between1-3 were classified as susceptible (Kasrawi, 1989).

The most recent scoring scale used was the 1–6 severity scale, where: 1 = healthy, no observable symptoms; 2 = very mild with slight yellowing and mosaic on top leaves and no leaf curling; 3 = mild yellowing, mosaic and/or slight leaf curling on youngest leaves, severe symptoms; 4 = moderate yellowing and/or leaf curling on the youngest (top) leaves; 5 = severe yellowing and blistering and/or severe leaf curling plus some leaf size reduction on the youngest leaves of the main stem and/or at least one branch; and 6 = very severe yellowing, blistering and/or very severe leaf curling, leaf deformation, leaf size reduction and stunting. The disease severity scoring was done every seven days after 17 and19 days old seedlings were exposed to the whiteflies (Hanson *et al*., 2016).

2.10.5.2 Detection of TYLCV in infected whitefly and tomato plants

In order to confirm that plants that do not show symptoms in the field are truly resistant, detection of TYLCV in plant samples is carried out. There are various ways of detecting TYLCV in infected plants. These include the use of Polymerase Chain Reaction (PCR) and Triple Antibody Sandwich and Enzyme-linked Immunosorbent assay (TAS - ELISA) techniques (Gajanandana *et al*., 2002).

PCR is a widely used technique in plant pathology for the detection of disease-causing organisms in infected plants, for cloning of genomic fragments of the pathogen (Henson and French, 1993), determination of the composition of pathogen population and genetic diversity of the pathogen (Robertson *et al*., 1991). PCR specificity is based on the set of oligonucleotide primers that are complementary to regions flanking the DNA sequence to be amplified. TYLCV DNA can be extracted from tissues in whiteflies squashed on the membrane, following hybridization with a radiolabeled (Navot *et al*., 1989) or with a chromogenic DNA probe (Zilberstein *et al*., 1989). TYLCV DNA can also be amplified from nucleic acids isolated from tomato plants and from individual whiteflies by PCR (Navot *et al*., 1992). In addition, TYLCV DNA can be amplified by combining both TAS-ELISA and PCR methods. PCR amplifies nucleic acids and can be used to overcome the many challenges associated with serological detection methods such as low titer of antigen, cross-reaction of antibodies with heterologous antigens and developmental or environmental regulation of antigen production, In the PCR detection of TYLCV in plants, different degenerate and specific primers have been used depending on the virus isolates and the availability of the primers. Table 2.1 lists primers used to differentiate isolates of TYLCV.

Table 2. 1: Primers and sequences for amplification of TYLCV DNA

Isolates	**Primers**	**nt position**	**Sequence (5'-3')**	**Size (bp)**
TYLCV	TYv2337	2337	ACGTAGGTCTTGACATCTGTTGAGCTC	634
	TYc138	188	AAGTGGGTCCCACATATTGCAAGAC	
TYLCV-Mld	TYm2664	2664	ATTGACCAAGATTTTTACACTTATCCC	316
	Tyc138	138	AAGTGGGTCCCACATATTGCAAGAC	
TYLCSV-ES	Almv2516	2516	TTTTATTTGTTGGTGTTTGTAGTTGAAG	433
	Almc115	115	ATATTGATTGGTTTTTTCAAACTTAGAAG	

Also, Osei *et al*. (2012) and Segbefia *et al.* (2015) used the primers in Table 2.2 for detection of TYLCV from symptomatic tomato leaf samples in Ghana. The last three primers have been developed for Tomato Yellow Leaf Curl Ghana Virus (TYLCGHV), Tomato Yellow Leaf Curl Kumasi Virus (TYLCKV) and Tomato Yellow Leaf Curl Virus Mali (TYLCMV). Again, Bang *et al*. (2014) used AV494/AC1048 and PTYc787/PTYc1121 in detecting TYLCV in infected tomato plants.

Table 2. 2: Primers and sequences for amplification of TYLCV DNA

Primers	**Sequence (5'-3')**	**Primer type**	**Reference**
PARc1496	GCAGGCCCACATYGTCTYCCNGT	Degenerate	Rojas *et al.* (1993)
PAL1v1978	AATACTGCAGGGCTTCTRTACATRGG		
AV494	GCCCATGTATAGAAAGCCAAG	Degenerate	Wyatt and Brown (1996)
AC1048	GGATTAGAGGCATGTGTACATG		
PTYc787	GTTCGATAATGAGCCCAG	Degenerate	Zhou *et al.* (2008) and Salati *et al.* (2002)
PTYc1121	ATGTAACAGAAACTCATG		
GHF	GCCCGAAAGCTTCGTTGTTTTCCCGCT	Specific	Osei *et al.* (2008)
GHR	ACGGATGGCCGCTTTGGGT ATTCG		
KF	GGACCCGGCGCACTATTTATGTTGGC	Specific	Osei *et al.* (2008)
KR	ACCCCATTACCCCAATACCA		
MF	TGGCCGCGCCCTTCCTTTTGT	Specific	Osei *et al.* (2008)
MR	ACCAATGGCTCCCCAAAGCGT		

2.10.6 Development of TYLCD resistance tomato in Ghana

TYLCD is a major tomato disease in Ghana and Africa as a whole and can cause massive yield loss (Osei *et al*., 2012). Considering the importance of TYLCD in Ghana, most of the research works focused on screening tomato accessions for TYLCD resistance. The USAID West African Regional Programme identified research on Virus resistance (VR) as a priority, and Ghana was included in seven members' regional investigation of tomato virus complex (Horna *et al*., 2006). The Agricultural Biotechnology Support Project II (ABSPII) aimed to improve agricultural production in developing countries through Biotechnology. The project was initiated in 2005 to address tomato production constraints in West Africa. This project was a partnership among researchers from AVRDC, Cornell University and University of California-Davis (UC Davis). The ABSII established the Regional Vegetable Germplasm Trailing Network that evaluated, 100 putatively TYLCD resistant tomato varieties that were adaptable to the growing conditions of West Africa which from 2005 through 2008. In the 2005-2006 growing, only 40 varieties were evaluated. The resistant varieties used for the entire trial were mainly F_1 hybrids since they were sourced from commercial seed companies and some breeding lines from breeding institutions. Based on the TYLCD scoring scale, at the end of the 2007-2008 multilocational trails, varieties such as Lety F_1 scored below 1, Yosra scored 1, Atak, Bybal and Gempride scored between 1.0 and 2.0 in Ghana (Navrongo and Technimanitia). The lower score was an indication of tolerance under the disease pressure. It was noted that the varieties suffered under farmers' field compared to research stations under comparable disease pressure. At the various trial locations, farmers-preferred Lety F_1, Yosra, Atak and Bybal. Due to the competitive nature of the tomato breeding industry in the developed world, some of the selected varieties were no longer in use in the countries where they were originally bred (Gordon, 2009).

Fifteen (15) tomato accessions (collected from AVRDC-Taiwan and CSIR - Crops Research Institute, Ghana) that have been reported to be resistant to TYLCD, as well as susceptible checks, were screened against the TYLCD in a greenhouse at the Kwame Nkrumah University of Science and Technology (KNUST) in Kumasi. These 15 accessions were later evaluated in the field at Afari (hot spot) in the Ashanti Region. The whiteflies used for the greenhouse inoculation were collected from infested tomato plants at Akumadan, Agogo and Afari. The incidence and severity of TYLCV were scored 30 days, 45 days and 60 days after transplanting using the 0-4 severity scale developed by Lapidot and Friedmann in 2002. At 60 days after transplanting in the greenhouse, accessions A2 (FLA456-4), G14 (WSP2F7 (3) PT.3) and G15 (WSP27F7 (3) PT.3) expressed moderate symptoms in terms of incidence of the TYLCV while accessions A8 (99S-C-39-20), A9 (H24), G13 (WS273.3LARGE) and G12 (WSP2F1PT.3) showed mild symptom of the disease. A1 (TY52), A3 (FLA478-6-3-0), A6 (TLB111), A7 (LA 1969) expressed slight severity symptoms of the disease. Accessions G11 (PIMPILIFOLIUM) and A1 (FLA505) had the lowest incidence compared to accessions A10 (CLN2026D), G13 (WS273.3LARGE) and A4 (FLA653-3-1-0) that had the highest incidence of TYLCV infection in the field. At 60 days after transplanting only accession, A1 (FLA505) showed no TYLCV (Osei *et al.*, 2010).

Again, thirty (30) accessions (including the 15 accessions that were screened in the greenhouse and the field in 2010) were screened against the local strains of the virus in Afari in the Ashanti Region. Some of these accessions were reported to be resistant in other countries. Only two accessions (Local Rano and Petomech-Ghana/France) out of the 30 accessions expressed mild symptoms while accessions WSP2F1pt.3 and Tomato Red Cloud expressed moderate symptoms after 60 days of transplanting. In order to confirm the resistance or susceptibility observed in the field, six viral detection primers were used to screen all the 30 tomato accessions. From the results obtained in that study, none of the primers amplified viral DNA in Tomato Red Cloud. For WSP2F1pt.3, only one of the six primers (PAL/PAR) amplified the viral DNA. Only MF/MR primer amplified the viral DNA in Local Roma. For Petomech (Ghana/France), two primers (GHF/GHR and KR/KF) amplified the viral DNA.

Again, between 2010 and 2011, seven (7) tomato varieties were grown in the fields in the University of Ghana and the Volta region of Ghana. The symptom expression of the varieties against the TYLCV was confirmed in the laboratory using some of the primers listed above in addition to Beta 01/02. From the field screening, it was found out that Burkina (obtained from farmers in the Volta region) had the highest TYLCD incidence, followed by Petomech and the susceptible check. However, Petomech expressed higher severity than Burkina. Both severity and incidence were lower in the hybrids with the exception of F_1Thorgal that showed no symptom. AC1048/AV494 detected the most viral DNA in the samples collected. The primer set T0302-F/T0302-R did not amplify the *Ty-2* gene in any of the varieties evaluated. However, Primer P6-25-F/P6-25-R amplified a band size of approximately 400 bp in F_1 Jaquar, F_1 Nadira and *S. Pimpinellifolium* (Ossom, 2012).

Between 2011 and 2012, a group of researchers also evaluated the susceptibility of ten (10) accessions to TYLCD under field conditions. The accessions include *Solanum Pimpinellifolium*, Wosowoso, Chery red, Roma, Hyb -1 (Wosowoso x *S. Pimpinellifolium*), Hyb - 2 (Roma x *S. Pimpinellifolium*), Hyb - 3 (Cherry red x *S. Pimpinellifolium*), BC-1[Wosowoso x (Wosowoso x *S. Pimpinellifolium*)], BC-2 [Roma x (Roma x *S. Pimpinellifolium*)] and BC-3 [C-Red x (C-red x *S. Pimpinellifolium*)]. The results from the phenotypic screening were verified with molecular markers. This work also deployed both triple antibody sandwich enzyme-linked immunosorbent assay (TAS - ELISA) and PCR method (using the primers in Table 2.2) for the TYLCV detection in order to recommend a better way of detecting TYLCV Virus in infected samples. A TAS-ELISA kit with a known TYLCV-infected Nicotiana benthamiana positive control was used for the study. The study confirmed the superior sensitivity of the PCR technique as a TYLCV detection method compared to the TAS – ELISA technique (Segbefia *et al*., 2015).

Recently, there was a phenotypic evaluation of 36 local tomato genotypes for resistance against TYLCD in two locations (University of Cape Coast and Asuansi) in Ghana. The results showed that five accessions (K005 - Petomec, K100 - Local 3, K213 -AVTO 9804, K116 - Ashanti 2 and K042 - Tomatose) out of the 36 genotypes were selected for mild severity. Among them, 2 genotypes showed severe symptoms (K027 - Local, K202 - AVTO 0102) and one genotype (LV - Fadzebegye) showed moderate severity. In order to confirm the infection or otherwise of the eight tomato accessions selected for mild and severe symptom expression, two of the viral detection primers (AV494/AC1048 and PTYv787/PTYc1121) were used for the detection of the virus in infected plant samples. (Asare-Bediako *et al*., 2017).

2.11 Conclusion

Significant progress has been made in improving fruit quality and the introgression of *Ty* resistant genes into cultivated tomato in advanced breeding programmes. These lines are available for further breeding and adaptation to various locations in the world. Many commercial varieties with different level of resistance have also been developed. However, more research is required particularly in breeding for location-specific resistance and pyramiding of multiple *Ty* resistant genes together with other important tomato disease resistance genes into commercial varieties. In Ghana, very little tomato research targeted fruit quality improvement. Although various screening of tomato accessions against the TYLCD have been carried out, more research must be carried out on identifying which of the TYLCD resistance genes is resistant to the local TYLCV strains and introgress those resistance genes into adapted varieties. Improving yielding and fruit quality together with TYLCD resistance will largely address the challenges of tomato production in Ghana.

CHAPTER THREE

3.0 Farmers' perception of Tomato Yellow Leaf Curl Disease (TYLCD) and its implication on tomato breeding

3.1 Introduction

Pests and diseases are the major biotic factors that affect tomato production in Ghana, and this together with other production constraints has resulted in Ghana's inability to meet the demand for tomato. TYLCD was reported to be the most devastating tomato disease in Ghana and can result in 100% yield loss (Osei *et al.*, 2012). Mitigating the impact of TYLCD on tomato yield can lead to an increase in tomato production.

Modern plant breeding programmes must be market-driven (Horna *et al.*, 2006) and since farmers are the direct beneficiaries of a breeding programme it is necessary to understand their perceptions of tomato production constraints especially to the most devastating disease, TYLCD. TYLCD is one of the three most important tomato diseases in the world (Hanson *et al.*, 2016).

Over the years, most tomato farmers in Ghana relied on seeds of imported tomato hybrids, improved pure lines and local pure lines. The imported pure lines and hybrids are not adapted to the Ghanaian growing conditions in Ghana whereas the locally-adapted pure lines have poor fruit colour, low brix, many seeds and high-water content (Robinson and Kolavalli, 2010). On the contrary, the tomato preferred by the market is expected to have deep red colour, higher brix, ability to withstand travel shock and have long shelf life. In order to successfully produce fruits with these market-preferred traits, the variety must be able to withstand the high incidence of pests and diseases in the growing areas.

Designing and implementation a breeding programme to address these challenges will require a better understanding of farmers' perceptions of the disease and fruit quality traits that must be considered in a breeding programme (Sperling *et al.*, 2001). A major approach to get farmers involved from the onset of the breeding programme is through Participatory Rural Appraisal (PRA).

Involving farmers at the start of the breeding programme enables them to own the project and makes adoption of a new variety very easy. The involvement of Ghanaian farmers in a breeding programme has become imperative following the recent report on farmers' rejection of seeds supplied by the government under the Ministry of Food and Agriculture flagship programme 'Planting for Food and Jobs programme'. The reason given by the farmers for the rejection is that the seeds, especially vegetables, are not adaptable to local conditions. In the case of tomato, the Agricultural Extension Agents (AEAs) were concerned that the tomato seeds being supplied will not withstand pests and diseases in the growing areas. Farmers resorted to using their own sources of seeds which are unimproved and low yielding. This obviously will defeat the objective of increasing production of some of the country's major crops in order to make the nation food secure (Gakpo, 2017).

The objectives of this study were to:

i) identify tomato production constraints of farmers in Ghana.

ii) determine farmers' perceptions or knowledge of the impact of TYLCD and strategies employed to control the disease.

iii) identify the set of traits preferred by farmers in tomato to guide the development of new varieties.

3.2 Material and Methods

3.2.1 Study area

Participatory Rural Appraisal (PRA) was carried out in three regions of Ghana where tomato is predominantly grown. These regions included the Ashanti Region (Akumadan), Brong Ahafo Region (Tuobodom and Benekrom) and the Upper East Region (Vea, Tono and Pwalugu). Akumadan is located in the Offinso North district in the deciduous forest zone of Ghana. Tuobodom is in the Techniman North district and Benekrom is within the Dormaa Municipality, which borders Côte D'Ivoire. Tuobodom and Benekrom are located in Transitional zones. The forest and transitional zones are characterized by bi-modal rainy seasons; April through June and September through November. Vea, Tono and Pwalugu are located in the Bongo, Kassena-Nankana and Talensi districts respectively. The Upper East Region is located in the Sudan Savannah Zone and bordered to the north by Burkina Faso. This zone is characterized by one rainy season from May/June to September/ October and the annual rainfall during the period is 800 and 1,100mm (GSS, 2013). Tono and Vea are within the Kassena Nankana East Municipality.

These communities were chosen in consultation with the Agricultural Extension Agents (AEAs) of the Ministry of Food and Agriculture (MoFA) and all the farmers selected were tomato farmers.

3.2.2 Selection of Farmers

Multistage sampling technique was deployed in the selection of the respondent for this study. The first stage involved purposive sampling of districts that were engaged in tomato production. Secondly, with the aid of the Agricultural Extension Agents (AEAs) at the various districts, six communities were purposively sampled based on the level of tomato production. With the assistance of the AEAs, experienced tomato farmers were selected for focal group discussion in three out of the six communities (one per Region). This was followed by questionnaire administration to farmers in all the 6 communities. Ten farms per community were randomly selected and visited to assess the incidence and severity of TYLCD. A total of one 107 tomato farmers participated in the PRA. The participants were selected at random regardless of their age, gender, experience in farming and their social status in the community.

3.2.3 Survey procedure and data analysis.

The PRA tools employed in the study were Focal Group Discussions (FGDs) and Key Informant (KI) interviews using a semi-structured questionnaire. FGD was held in each district, followed by semi-structured questionnaire administration and farm visit. The questionnaire is presented in Appendix. Each FGD consisted of 20 tomato farmers and 10 farms were subsequently visited. Farmers were not given prior information on TYLCD hence topics discussed included the tomato variety being grown in the community, preferred characteristics of these varieties as well as production and marketing constraints. This was to avoid any biases in their response. To further understand specific issues, a formal survey was carried out involving 107 farmers (including some of the farmers who participated in the FGD). The number of questionnaires administered per locality was 22 in Ashanti Region, 40 in Brong Ahafo Region and 45 in Upper East Region. The number of tomato farmers differed for the communities. The PRA was carried out during the dry season because of the high prevalence of TYLCD during that period. Appiah *et al*. (2012) reported high prevalence of white fly during the dry season. In the Upper East Region, farms were visited during the fruiting stage (February 2016) of the plants. In the Ashanti and Brong Ahafo Regions, most of the farms were visited immediately after transplanting (September 2016) and a follow-up visit was made at the fruiting stage (November 2016). In each location, 10 farms were visited and the incidence and severity of TYLCD were scored. Farmers identified various diseases with the aid of a publication titled ‘A visual guide: Tomato foliage, stem and Root problems’ (Missouri Botanical Garden).

3.2.4 Data Analysis

Statistical Package for Social Sciences (SPSS) version 21 was used to analyze the data collected from the administered questionnaire. Descriptive statistics such as frequency, percentages and charts were used to describe the attributes of the variables collected.

3.3 Results

3.3.1 Focal Group Discussion

From the Focal group discussion in the Ashanti Region (Akumadan), it was established that the tomato varieties grown were Petomech, Power Rano, Akoma and Konkon. According to the farmers, Petomech was preferred because it had a relatively long shelf life and for the other varieties it was due to higher yields. These varieties took about 80 days to mature when the weather was favourable. Akumadan has two tomato growing seasons due to the bimodal rainfall pattern in the region. The first season is from February to May and the second season is from August to November. From the FGD, it was realized that the farmers were aware of the presence of Tomato Yellow Leaf Curl Disease in their farms. The local name given to this disease was ‘anointing’. The name was derived from the symptoms they observed in the fields. According to them, an infected plant looked yellow which can be likened to the colour of anointing oil used for spiritual purposes and the leaves then cupped as if raising one’s hands up in preparedness to receive blessings; hence the name ‘anointing.’

The farmers were of the view that there was no control for the disease. The farmers also mentioned other diseases like root-knot nematode and black spot as well as pests such as whiteflies and caterpillar.

The farmers indicated that tomato is imported from Burkina Faso into markets in Ghana from December to March when there is no production in Akumadan. They also alluded to the fact that Burkina Faso uses irrigation for production during that dry season.

Tuobodom was one of the two communities visited in the Brong Ahafo Region and the only tomato variety grown there was Power. The variety is known to show good performance in the area. According to the farmers, any other tomato variety including Petomech produced very tiny non-marketable fruits and they attributed it to the nature of their soil. According to them, Power takes 60-80 to reach maturity. The tomato-growing period in Tuobodom is similar to that of Akumadan. They also mentioned a number of diseases in the area and these included TYLCD, root-knot nematodes, damping off and Black Spot. Damping off was observed in most of their nurseries during our farm visits. At the nursery, seeds were broadcast and a large number of the seedlings were dying. The farmers described the symptoms of TYLCD and considered it as the most devastating disease in the area. The local name given to the TYLCD was ‘Mathwo’. This name was attributed to the symptoms since infected leaves were reduced in size, hardened and the stems were stunted. Some pests mentioned were caterpillar (locally called ‘Twenwomutae’), cricket and red mite. According to the farmers present at the FGD, the season during the PRA was the first time they were experiencing a massive outbreak of red mites.

Farmers in Tuobodom also complained about unstable prices for their produce and varying box sizes used by marketers in buying their produce and therefore called for standardization. Farmers in Tuobodom believed that tomato imported from Burkina Faso had long shelf life and reiterated their inability to farm in the dry season was because of lack of irrigation facilities.

According to the farmers in Dormaa, they cultivated many improved tomato varieties. The choice of a variety depended on the performance of the variety in the previous season. Hence, a different variety could be grown in successive season based on the performance of the variety in the previous year. Farmers claimed they produce premium tomato because they use improved seeds whereas the other two communities mostly used saved seeds. They also had well-raised nurseries and all tomato plants were staked. The farmers practiced shifting cultivation because of the availability of land. Dormaa also has two growing seasons; the first season is from March to June and the second from August to October. They indicated that TYLCD used to be a problem in the area. They did not have problem with the Ghana-Burkina Faso tomato trade because they are engaged in Ghana-Côte D'Ivoire tomato trade

The three major tomato growing areas in the Upper East Region namely Pwalugu, Vea and Tono cultivated Petomech, UC 82, Tropimec and 'No name' (likely to be Petomech). The farmers' choice of variety was determined by the preference of the market women. According to the farmers, these varieties are high yielding and have long shelf life. The first planting season is from September-February and the second is from November-March. The harvesting period partly coincides with that of Burkina Faso. Farmers were unhappy with Ghana-Burkina Faso tomato trade because the harvest period coincides with that of Burkina Faso. The farmers alleged that the market women preferred to go to Burkina Faso because they take the opportunity to trade in other commodities. According to them the market women buy items from Ghana and conceal them under the tomato boxes and these items are sold in Burkina Faso to earn West African CFA franc and in return buy tomato back to Ghana. Some of the farmers also said because they did not get government support, they are unable to produce in larger quantities and the quality of their tomato was not as good as tomato from Burkina Faso. This was attributed to pests and diseases, particularly TYLCD. Most farmers in Pwalugu were growing onions because of poor market as well as pests and diseases. In Tono, some farms outside the irrigation plots were prone to animal grazing.

3.3.2 Demographic characteristics of questionnaire respondents

The results showed that 74% of the 107 respondents were men and 26% were women (Table 3.1). The respondents belonged to different age categories with 39% of them between 31 and 40 years, 31% were between 41 and 50 years and 16% were above 50. A few farmers (3%) had tertiary education with the majority (58%) of them having primary and Junior High School education. Fifteen percent (15%) had secondary school certificate while 22% of the farmers did not have any formal education.

Table 3. 1: Gender, age and educational background of tomato farmers from three regions in Ghana

Attribute	**Percentage (%) of respondents**
Sex	
a. Male	74
b. Female	26
Age (Years)	
a. 18 - 30	14
b. 31 - 40	39
c. 41 - 50	31
d. Above 50 years	16
Educational level of farmers	
a. No formal	22
b. Primary	33
c. JHS	25
d. Secondary	15
e. Technical/Vocational	2
f. Tertiary	3

3.3.3 Tomato Production

Most of the farmers (86%) have been growing tomato for upwards of 6 years while 1% of the farmers had less than one year's experience in tomato cultivation (Table 3.2). Majority (63%) of the respondents owned 1-3 acres of tomato farm, 13% owned 4-6 acres of tomato farm and 6% owned between 7 and 14 acres. A few (1%) of the farmers owned 19 acres and above.

Table 3. 2: Number of years of experience in tomato farming and size of farm owned by farmers in six tomato-farming communities three regions in Ghana

Years of farming	**Percentage (%) of respondents**
a. Less than a year	1
b. 1-5 years	13
c. 6-10 years	43
d. more than 10 years	43
Size of farm	
a. >1 acre	16
b. 1-3 acres	63
c. 4 – 6 acres	13
d. 7 – 10 acres	6
e. 11 - 14 acres	1
f. > 19	1

The respondents obtained their seeds from different sources. Majority (41 %) of respondents obtained seeds from Agro-seed shops, 36% used saved seeds and the rest of the respondents obtained seeds from other sources (Figure 3.1).

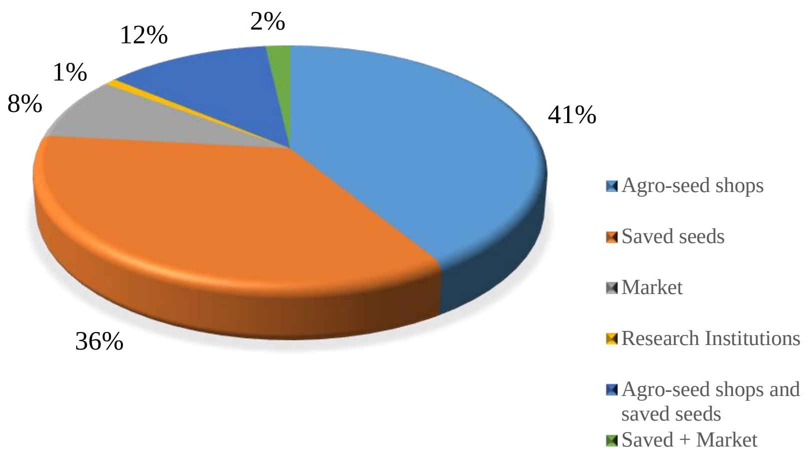

Figure 3. 1: Distribution of respondents with respect to source of seeds for tomato production

About (60%) of the farmers purchased new seeds every season. Some 37% on the other hand only used saved seeds. Only one percent obtained new seeds when a new variety is introduced (Figure 3.2).

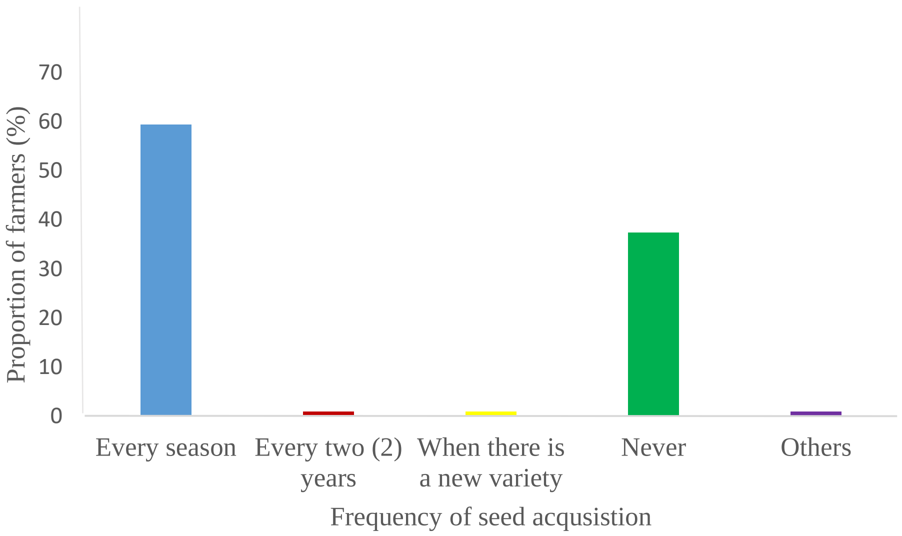

Figure 3. 2: Frequency of seed acquisition for tomato cultivation among respondents in six farming communities in three regions in Ghana

3.3.4 Constraints to tomato production

In the six communities, each respondent listed a varied number of constraints to tomato production in Ghana (Table 3.3). Across the three Regions, farmers perceived pests and diseases as the most important constraints to tomato production. Pest and diseases were scored 91%, 100% and 96% in Ashanti, Brong Ahafo and Upper East Regions respectively. For drought, 90% of respondents in the Brong Ahafo Region, indicated this as a major production constraint, followed closely by Ashanti Region (81%) and 31% of farmers in the Upper East Region. Again, 90% of respondents in Brong Ahafo Region perceived the cost of fertilizer and insecticides as high and a constraint to production, the second highest being in the Upper East Region (78%) and the least number of respondents indicating cost of agrochemicals as a production constraint was observed in the Ashanti Region (59%). All respondents in the Brong Ahafo Region viewed inadequate financing as a major production challenge, followed by Ashanti Region (86%) and lastly Upper East Region with 68% of respondents.

Lack of technical support from Agricultural Extension Agents had the lowest number of respondents classifying it as a major production constraint in the three regions. Three percent (3%) of farmers in Brong Ahafo Region, 23% in Ashanti Region and 24% in Upper East Region viewed extension services as a major challenge. Many farmers (82%) in the Ashanti Region perceived low-quality seeds as a major challenge to production, whereas only 43% of respondents in the Brong Ahafo and 18% in the Upper East Region considered it as a major production constraint.

Table 3. 3: Perception of tomato farmers of the key production constraints in six farming communities in three regions in Ghana

Constraints	**Ashanti Region**	**Brong Ahafo Region**	**Upper East Region**
Drought	81%	90%	31%
High cost of fertilizer and insecticides	59%	90%	78%
Inadequate finance	86%	100	68%
Lack of technical support from AEAs	23%	3%	24%
Low-quality seeds	82%	43%	18%
Pests and diseases	91%	100%	96%
Others	50	48%	56%

3.3.5 Perception of biotic constraints to tomato production among farmers

Many farmers (45%) in the Ashanti Region ranked caterpillar as the most important pest (Table 3.4). For the Brong Ahafo Region, 25% of the farmers ranked caterpillar as the most important pest. In the Upper East Region, 47% of the farmers ranked whitefly as the most important pest.

Table 3. 4: Perception of tomato farmers of the importance of weeds and insect pests as constraints to tomato cultivation in three regions in Ghana

Pests	**Ranking per Region (%)**														
	Ashanti Region					**Brong Ahafo Region**					**Upper East Region**				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Grasshoppers	-	-	18	-	-	15	20	20	13	-	4	7	9	7	-
Caterpillar	45	32	5	-	-	25	20	28	5	-	27	29	18	-	-
Weeds	-	-	14	-	-	3	-	-	-	-	-	-	-	-	-
Whiteflies	5	-	-	-	-	3	5	5	5		47	24	9	4	-
Butterfly	-	-	-	-	-	-	-	-	-		-	4	2	2	-
Stem borer	-	-	-	-	-	-	-	-	-		-	2	-	-	-
Others	-	9	5	5		30	23	18	3			13	16	9	4

Note: 1-Most important; 2-Very important; 3-important; 4-Less important; 5-Least important; 6-Not important

Tomato Yellow Leaf Curl Disease (TYLCD) was ranked as the most important disease of tomato in all the three regions (Table 3.5). Many farmers (68%) in Ashanti Region ranked

TYLCD as the most important disease of tomato compared to 18% of farmers in the Brong Ahafo Region. Fusarium wilt was perceived to be present in only the Ashanti Region.

Table 3. 5: Perception of tomato farmers of the importance of diseases of tomato in three regions in Ghana

Disease	**Ranking per Region (%)**														
	Ashanti Region					**Brong Ahafo Region**					**Upper East Region**				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Damping off	-	-	-	-	-	5	23	13	10	-	-	4	2	-	-
Early Blight	14	41	14	18	-	3	3		8	-	-	-	-	-	-
Late Blight	-	-	-	-	-	-	3	8	8	-	-	-	-	-	-
Fusarium Wilt	14	5	14	-	-	-	-	-	-	-	-	-	-	-	-
Bacteria Wilt	-	-	-	-	-	-	-	-	-	-	4	7	9	-	-
TYLCD	**68**	23	5			**18**	5	8	3	3	**58**	9	7	2	
Blossom end rot	5	14	-	-	-	-	-	-	-	-	2	2	-	-	-
Nematode	-	-	-	-	-	5	3	-	-	-	2	2	2	-	-
Others	-	14		5		60	3	18	-	-	11	47	9	-	-

Note: 1-Most important; 2-Very important; 3-important; 4-Less important; 5-Least important; 6-Not important

3.3.6 Farmers' knowledge, perception and experiences concerning TYLCD prevalence

All the respondents except 5% were aware of TYLCD (Figure 3.3). Eighty-four (84) % of respondent indicated that they observed the disease on their farms and 1% of farmers read it in a scientific publication as well as observed it on the farm. A total of 76% of the respondents interviewed did not know about the cause of TYLCD and 6% of respondents stated that TYLCD is caused by whitefly (Table 3.6).

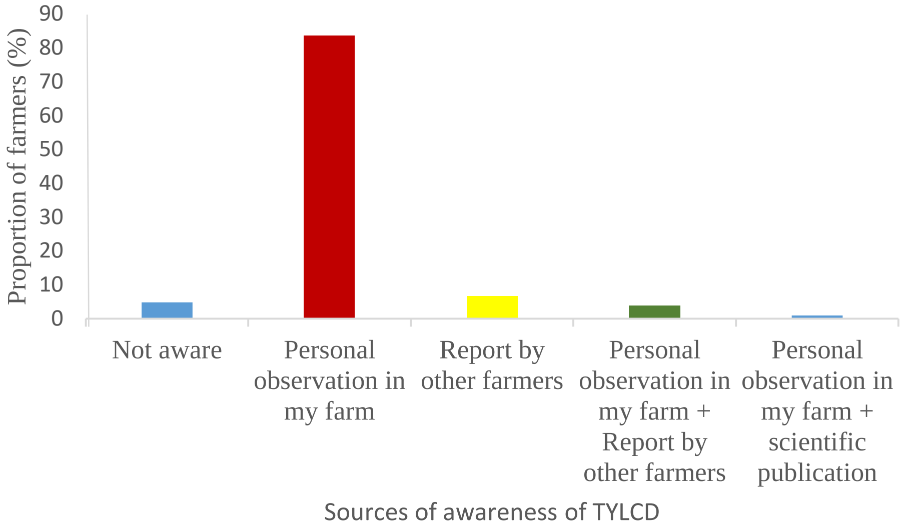

Figure 3. 3: Sources of Awareness of TYLCD among tomato farmers in six communities in three regions in Ghana.

Table 3. 6: Farmers' perception of the vector of Tomato Yellow Leaf Curl Disease in six tomato farming communities in three regions in Ghana

Causes of TYLCD	Percent (%) of respondents
Don't know	76
Aphid	1
Red mite	3
Whitefly	6
Caterpillar	6
Grasshopper	2
Whitefly and Grasshopper	1
Caterpillar and Grasshopper	2
Whitefly and Caterpillar	1
Others	2

Figure 3.4 shows that 40% of the respondents indicated pepper as an alternative host of whitefly. Some 25% of the respondents also indicated that pepper and eggplants were alternate hosts of the vector and 7% also mentioned eggplant as an alternate host of the vector. Nine percent (9%) of respondents did not have an idea about the alternate host of the vector.

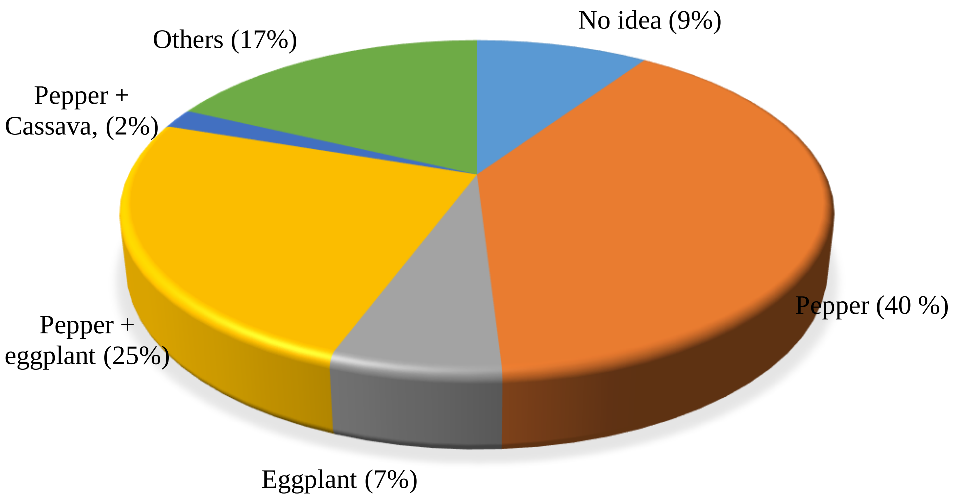

Figure 3. 4: Proportion of respondents among tomato farmers in six tomato farming communities in three regions in Ghana that perceived various crop plants as alternative host of the whitefly

Respondents expressed the symptoms of TYLCD they observed in their fields and 45% indicated yellowing and curling of leaves, 22% mentioned yellowing of leaves and 10% indicated curling and cupping as well as yellowing and reduction of leaves (Figure 3.5).

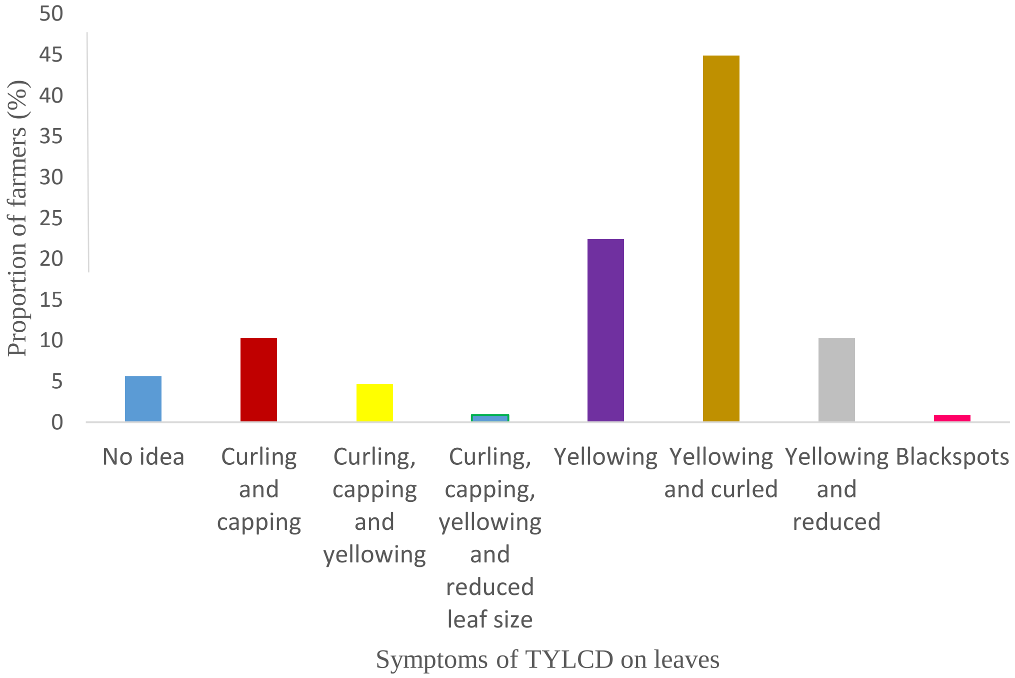

Figure 3. 5: Proportion of respondents that ascribed various leaf symptoms of infection by TYLCD in six tomato farming communities in three regions in Ghana

Respondents also described symptoms of TYLCD on infected stems (Table 3.7). Majority (46%) of the respondents indicated stunted growth, 18% indicated hardened stem, 16% indicated both stunted and hardened stem, 14% did not have an idea and the rest of the respondents indicated rots and death among others.

Table 3. 7: Farmers' knowledge of the symptoms of Tomato Yellow Leaf Curl Disease on stem of tomato plant in three regions in Ghana

Symptoms on stems	Percent (%) of respondents
no idea	14
Stunted growth	46
Hardened stem	18
Stunted growth and hardened stem	16
Rots	1
Death	1
Others	4

Majority of the respondents (60%) indicated that the plants expressed symptoms after 3-4 weeks after transplanting (Table 3.8). Some 14% of respondents also indicated 1-2 weeks after transplanting. The rest of the respondents indicated 5-6 weeks after transplanting, at the nursery and others.

Table 3. 8: Farmers' perception of the tomato stage of growth of first symptom expression following TYLCD infection in the field in three regions in Ghana

Period of first symptom	Frequency	Percent (%) of respondents
0-Absent	1	1
At the nursery	8	7
1-2 weeks after transplanting	15	14
3-4 weeks after transplanting	64	60
5-6 weeks after transplanting	10	9
7 weeks and above	2	2
Others	7	7

Farmers' perception of varieties of tomato that were commonly affected by the TYLCD in their communities is represented in Figure 3.6. Twenty-eight percent (28%) of respondents indicated all varieties, whereas 26% and 10% specified Petomech and Power Rano, respectively. Other varieties mentioned were Tropimec, 'No name' and UC 82.

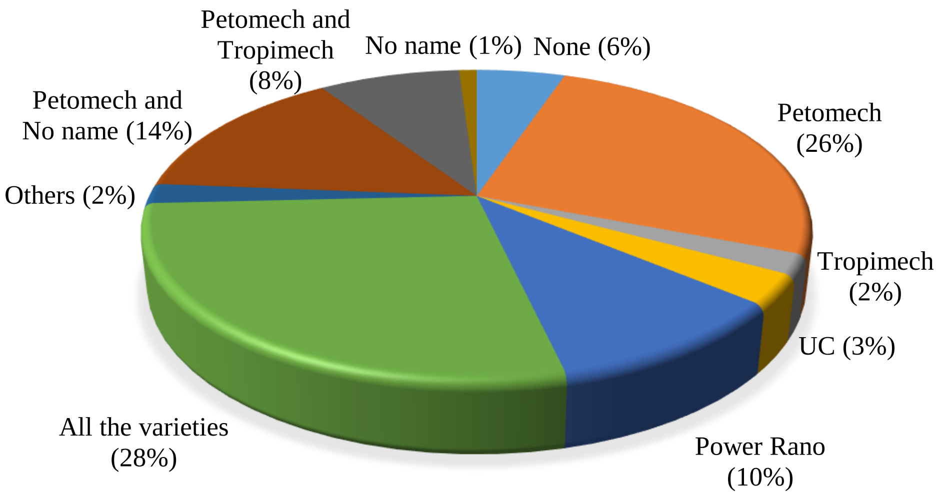

Figure 3. 6: Proportion of farmers in six communities in three regions in Ghana that identified particular tomato varieties as susceptible to TYLCD

Most of the (58%) respondents indicated that the disease affected their farm every season, 22% of respondents also stated yearly and a few of the respondents (5%) indicated every other year (Figure 3.7).

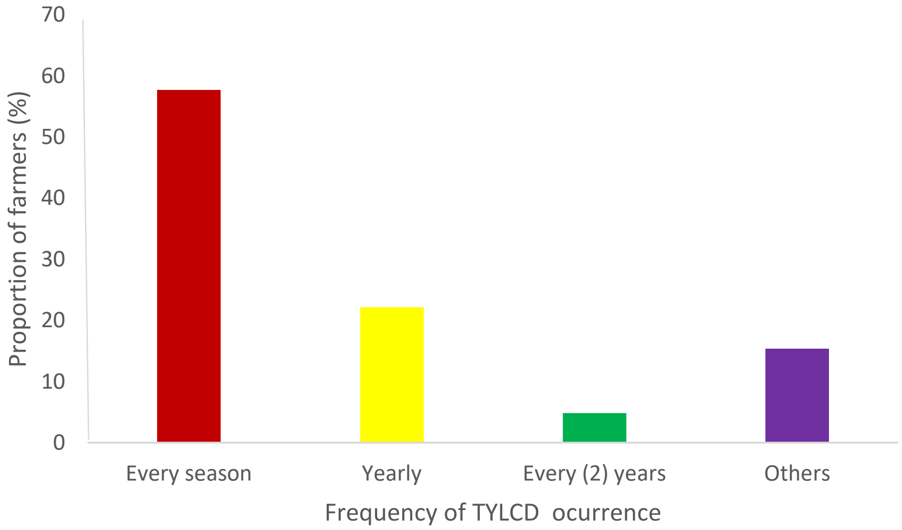

Figure 3. 7: Frequency of TYLCD occurrence in farmers' field based on respondents in six tomato growing communities in three regions in Ghana**.**

With regards to the level of incidence of TYLCD that farmers experienced annually, 36% of respondents stated that between 25% and 50% of their farms got infected by the TYLCD, 25% indicated that between 50% and 75% (Figure 3.8). Twenty percent (20%) of respondents indicated that the TYLCD disease infected less than 25% whereas 6% of respondents noted that 100% incidence.

A total of 38 of the farmers experienced yield loss between 25%; and 50% and 26 of the farmers also experienced yield loss of between 50% and 75%. Eighteen and 6 of farmers lose up to 25% and 100%, respectively, of their yield due to the disease (Table 3.9).

Table 3.9: Farmers' perception of estimated yield loss caused by TYLCD in three regions in Ghana

No. of farmers	Description of yield loss
18	Up to 25% yield loss
38	Between 25% and 50% yield loss
26	Between 50% and 75%
19	Between 75% and 100% yield loss
6	100% yield loss

3.3.6.1. Farmers' perception of the control of TYLCD

According to 27% of the respondents, there was no control for TYLCD, 58% indicated chemical spraying. Two percent (2%) indicated separating new field from existing tomato farms. In addition, 1% each indicated practicing crop rotation, and farm sanitation as methods of TYLCD control (Table 3.10).

Table 3. 10: Measures adopted by farmers to control TYLCD

Control measures	Percent (%) of respondents
No control	27
Separate old field from new field	2
Practicing crop rotation	1
Proper farm sanitation	1
Chemical spraying	58
Combination of at least two practices	5
Roguing	6

Majority of the respondents (67) had no idea of the use of TYLCD resistant varieties as a means of controlling TYLCD (Figure 3.8). A total of 30% of respondents knew TYLCD resistant varieties could be used as a means of controlling TYLCD.

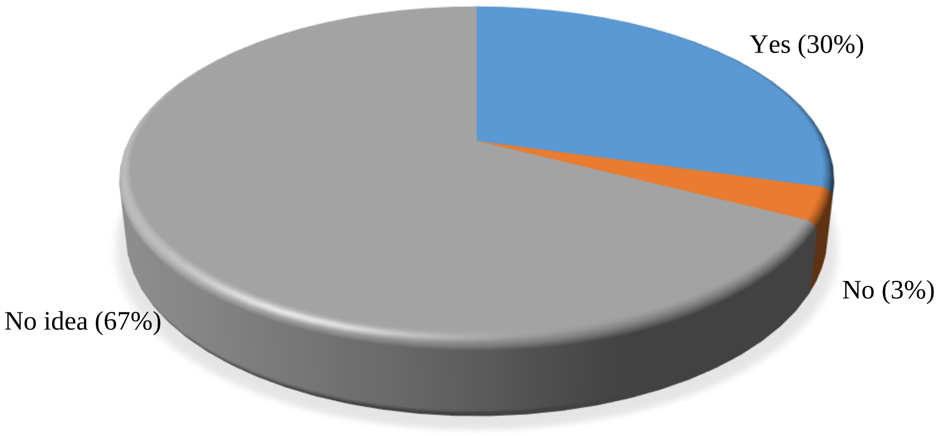

Figure 3. 8: Farmers' knowledge about the use of resistant TYLCD variety as a means of control

Tomato farmers in Ashanti Region wanted breeders to consider yield as the most important trait in the development of a new variety (Table 3.11). TYLCD resistance or tolerance should be considered as the second most important trait with shelf life as the third most important trait in developing a new variety and heat tolerance was the least important trait to them. Tomato farmers in Brong Ahafo Region ranked heat tolerance as the most important trait to be considered by breeders in the development of a new variety. In Upper East, TYLCD was the most important trait that must be considered by breeders. This should be followed by yield and then shelf life.

Table 3. 11: Farmers ranking of preferred traits that must be considered in breeding a new tomato variety

Traits	Ashanti Region	Brong Ahafo Region	Upper East Region
Yield	1	3	2
Shelf life	3	5	3
Big Fruit	4	4	6
Drought Tolerance	5	2	5
Heat Tolerant	6	1	4
TYLCD resistance	2	6	1

Note: 1-Most important; 2-Very important; 3-important; 4-Less important; 5-Least important; 6-Not important

3.3.7 Incidence and severity score of TYLCD in the regions studied

All the three villages in Upper East recorded 100% incidence, however, Dormaa recorded 1% TYLCD incidence (Table 3.12). Tono had the highest TYLCD severity score of 4 and Dorma had the least severity score of 2.

Table 3. 12: TYLCD incidence and severity scores in 10 farms of six tomato farming communities in each of the three regions in Ghana based on a rapid field survey

Village	Incidence (%)	Mean severity score
Akumadan	84	3
Tuodobom	85	3
Dormaa	1	2
Vea,	100	3
Tono	100	4
Pwalugu	100	3

Severity score: Healthy, 2 – Very Mild, 3 - Mild, 4 – Moderate, 5 – Severe, 6-Very Severe

3.4 Discussion

3.4.1 Tomato production constraints

The farmers mentioned a number of tomato production constraints. The most important tomato production constraints in all the three regions were pests and diseases. Other constraints peculiar to a particular locality were also mentioned. Farmers in Ashanti and Brong Ahafo mentioned drought as one of the constraints to tomato production. High cost of fertilizer and insecticides was of concern to farmers in Brong Ahafo and Upper East as compared to Ashanti Region. Lack of adequate finance was an absolute problem in Brong Ahafo relative to Ashanti and Upper East Region. This finding is consistent with Robinson and Kolavalli (2010) who reported high per unit input price, pests and diseases as reasons for low productivity of tomato in Ghana.

Paramount among the diseases of tomato in the six tomato growing communities was the TYLCD. In this study, 84% of the farmers were aware of the TYLCD and Asare-Bediako *et al*. (2017), reported 92.6% of the farmers were aware of TYLCD. The farmers described different TYLCD symptoms on leaves which included yellowing, curling, cupping and reduced leaf size. They also mentioned symptoms associated with the stem such as stunted growth, hardened stem and eventual death of the plant. With the exception of hardened stems, all the symptoms described by the farmers are characteristics of TYLCD (Hanson *et al*., 2016; Yan *et al*., 2018). Majority (60%) of the farmers observed the symptoms of TYLCD 3-4 weeks after transplanting and others also observed it as early as one week after transplanting. Mensah-Wonkyi (2014) reported 78% of the farmers observed the symptoms in their farms during the flowering stage. TYLCD infection at an early stage of the plant development can lead to total yield loss (Levy and Lapidot, 2008).

Majority of the farmers indicated that TYLCD affected their farm every season and others indicated yearly. This gives an indication of the prevalence of the disease in the various communities. According to the farmers, TYLCD affects more than half of the entire farm and in some cases, the disease affects the entire farm. This infection leads to more than 50% yield loss or total yield loss. Aare-Bediako *et al*. (2017) reported in a survey carried out in the Central Region, farmers lost 10% to 40% of their yield due to TYLCD infection.

Although many respondents indicated that TYLCD was the most important problem of tomato, a few indicated that whitefly transmits the disease. This is not surprising since majority of the respondents said they did not know the cause of the disease. This is consistent with the work of Mensah-Wonkyi (2014) in the Central Region where majority of the farmers were aware of TYLCD but did not know the vector of the virus. Some farmers attributed it to climatic factors. In the present study, many farmers attributed it to other pests such as aphids, red mite, and caterpillar. The whitefly is the vector that transmits Tomato Yellow Leaf Curl Virus (TYLCV) that causes TYLCD.

Farmers were aware of alternate host of whiteflies in their fields. Some of the alternate hosts listed were pepper, garden egg and cassava. Although tomato serves as the primary host for the whitefly or virus, there are other hosts. These include cucumbers, cotton, eggplants, potatoes, tobacco, beans, peppers and some weeds among over 300 species within 63 families (Mound and Halsey, 1978).

Albeit TYLCD was the most devastating, some farmers believed there is no control. Some farmers resorted to chemical spraying in an attempt to control it. Asare-Bediako *et al*. (2017) also reported that 60.4% of farmers managed TYLCD by spraying. The use of chemicals has been partially effective because the vector has to be eliminated before it transmits the virus. There are also concerns that the vector may develop pesticide resistance (Lapidot, 2003).

Majority of the farmers have no idea that Tomato Yellow Leaf Curl Disease Resistant tomato cultivars exist and can be used to control the disease.

Many farmers indicated none of the variety they grow presently is resistant or tolerant to the disease. The farmers in all the communities studied suggested that high yield, TYLCD resistance and long shelf life must be considered in developing a new variety.

3.4.2 Incidence and Severity of TYLCD

TYLCD was present in all the areas studied. In the middle belt, TYLCD was more severe in Tuobodom than Akumadan and in the Upper belt; Vea recorded the lowest severity score and Tono recorded the highest severity score. High incidence of TYLCD was reported in Ashanti Region and Upper East Region (Horna *et al*., 2006; Osei 2013). The severity recorded in this study was lower due to the growth stage of the plants during the survey period. This is consistent with the results of Asare-Bediako *et al*. (2017) who reported that even though TYLCD was present in the study area, the severity was low.

3.5 Conclusion

This study identified major tomato production constraints and farmers' preferred traits that must be taken into consideration in developing a new variety. There were several tomato production constraints in Ghana. However, the TYLCD was the major biotic constraint to tomato production in the communities studied. All the farmers were aware of the existence and the devastating nature of TYLCD. Majority of the farmers estimated between 50-100% of yield loss due to TYLCD. Farmers relied on pesticides in an attempt to control TYLCD. Tomato farmers in all the communities studied ranked yield, TYLCD resistance and long shelf life as important traits that must be considered in developing a new variety.

CHAPTER FOUR

4.0 Diversity analysis of tomato accessions based on morphological traits and SNPs markers

4.1 Introduction

Tomato is an important ingredient in most Ghanaian meals served in households and hotels. Notwithstanding the importance of tomato in Ghana, cultivation over the years has failed to reach its potential (Robinson and Kolavalli, 2010). Ghana produced 366,772 tonnes of tomato in 2016 with an estimated yield of 7.8 t/ha compared to 10.2 t/ha in Burkina Faso, 15.59 t/ha across the African continent and 37 t/ha world average output (FAOSTAT, 2016).

The low yields recorded in Ghana is attributed to non-availability of quality tomato varieties adapted to local production environment, inadequate irrigation facilities as well as prevalence of pests and diseases. Tomato production in Ghana is dominated by imported pure lines, unadaptable hybrids, farmer-selected cultivars as well as local landraces (Osei *et al*., 2014; MoFA, 2018). Many farmers still prefer to grow local cultivars because of their adaptability to local growing conditions. However, these varieties have high water content, many seeds and poor colour (Robinson and Kollavalli, 2010).

In order to alleviate the current challenges of the tomato industry in Ghana, there is the need for a comprehensive breeding programme that will address these important constraints of tomato production in Ghana. To implement this comprehensive breeding programme, analysis of the diversity in the country's tomato germplasm collection as well as introduced lines is a prerequisite (Mohammadi and Prasanna, 2003; Ziaf *et al*, 2016).

There have been series of germplasm collections before 1980s to 2012 by the Plant Genetic Resources Research Institute of the Council for Scientific and Industrial Research (CSIR-PGRRI) and National Agriculture Research Programme from all the ten regions in Ghana. The 2012 tomato germplasm collection extended to two districts in Burkina Faso (Kougoussi and

Yako), Asian Vegetable Research Development Centre (AVRDC), the Rural Development Administration in Taiwan, National Institute of Horticulture and Herbal Science (NIHHS) and the Republic of Korea (Osei *et al*., 2013). Some of this assembled germplasm together with imported cultivars have been evaluated for morphological and agronomic traits (Blay *et al*., 1999; Kugblenu *et al.,* 2013, Osei *et al.*, 2013; Osei *et al*., 2015). Morphological traits are simple approaches to estimate genetic diversity and cultivar development (Fufa *et al*., 2005). However, morphological traits are prone to phenotypic plasticity (Govindaraj *et al.*, 2015).

The current development in molecular studies has become important in the establishment of diversity in a germplasm pool. Gongolee (2014) evaluated some introduced fresh market tomato for genetic variability and adaptability in Ghana using Simple Sequence Repeat (SSR) markers. Although, SSR has been used to establish genetic variability and population structure in tomato and other species (Aguirre *et al*., 2016), SNPs have become the choice of DNA marker for high throughput analysis of plants due to availability of cost-effective, accurate and fast genotyping assays (Zhao *et al*., 2010 and Corrado *et al*., 2013).

This study sought to establish the genetic diversity among the assembled germplasm based on morphological traits valued by the fresh tomato market and use SNP markers to characterize the collection to provide baseline information for further breeding of the crop. The objective of this study was to determine the genetic diversity of tomato germplasm in Ghana based on important traits for development of commercial varieties.

4.2 Materials and Methods

4.2.1 Genetic Materials and Experimental site

A total of 123 accessions (Table S1) consisting of cultivated landraces, commercial varieties and breeding lines used for the study were obtained from the Plant Genetic Resource Research Institute (PGRRI) of the Council for Scientific and Industrial Research (CSIR), Forest and

Horticultural Crops Research Centre (FOHCREC), of the University of Ghana (Legon), Department of Crop Science, University of Ghana (Legon), Biotechnology and Nuclear Agriculture Research Institute (BNARI), Tomato Genetic Research Centre, University of California (TGRC, UC), Syngenta Seed (Switzerland), Wienco Ghana Limited, Technisem (France) and Agrimat Limited (Ghana). The accessions from FOHCREC, Department of Crop Science, Syngenta seed, Wienco and Agrimat were improved lines and varieties. The field experiment was carried out at the University of Ghana Farms between latitude 5° 38 45 N and longitude 00^{o} 11 13 E. The soil type was Haatso Series and average rainfall was 809 mm. The minimum temperature was 23.8 °C and the maximum temperature was 31.2 °C.

Of the 123 tomato accessions grown in the field, 96 of them were used for the SNPs based genotyping. Leaf discs were obtained from freshly harvested leaf of selected plant of each accession for genotyping. The Genotyping was performed at LGC Genomics, UK using the core set of 384 tomato KASP assays developed during the Solanaceae Coordinated Agricultural Project (Sim *et al*., 2013). These markers were evenly distributed across the tomato genome, based on both genetic and physical maps.

4.2.2 Experimental Design and Field Layout

The experiment was laid out in an augmented design. There were 11 blocks and 11 accessions in each block with 2 standard varieties repeated in each block. The two standard varieties were Power Rano and Peto Mech. These varieties were used as standards based on their wide cultivation by farmers within the country. Two-row plots were used with inter-row spacing of rows 1m and intra-row spacing of 0.5 m. The row length was 4.5 m and there were 10 plants per row.

4.2.3 Nursery and Agronomic practices

Tomato seeds of each accession were planted in nursery trays on 1st April 2016. Three seeds were planted per cell of the nursing tray and were later thinned to one seedling per cell. Thirty-day-old seedlings were transplanted to the field. Watering was done when necessary. At two weeks after transplanting, N.P.K. 15-151-15 was applied at the rate of 8-10g per plant. At four weeks after transplanting, Sulphate of Ammonia was applied at the rate of 5g per plant. Calcium nitrate (Fertigation grade) was applied at fruiting at the rate of 150 g/L.

4.2.4 Data Collection

Vegetative, reproductive and fruit quality traits were recorded based on the International Plant Genetic Resources Institute (IPGRI, 1996) tomato descriptor and the International Union for the Protection of New Varieties (UPOV, 2011) tomato descriptor.

4.2.4.1 Morphological Parameters

1. The plants of each accession were observed and scored as either determinate, semi-determinate and indeterminate

2. Foliage density: The foliage of plants per accession were visualized and scored as sparse, intermediate and dense.

3. Leaf coverage: The leaf coverage was scored based on visual observation of how the fruits of the plants were covered with leaves and recorded as exposed, intermediate or exposed.

4.2.4.2 Phenological parameters

1. Days to first flowering: Number of days from transplanting to the date the first plant had an open flower was recorded
2. Days to first maturity: the number of days from transplanting to the date the first fruit turned red.

3. Reproductive duration was estimated as the difference between days to first flowering and days to first maturity.

4.2.4.3 Yield parameters

1. Number of fruits per plant: The total number of fruits harvested from each accession was counted.
2. Fruit weight: The total weight of fruits per harvest was recorded and divided by the number of fruits
3. Yield: Total weight of fruits per plot in g was converted to tonnes per hectare.

4.2.4.4 Fruit quality Parameters

1. Fruit green shoulders: The absence or presence of green shoulder on fruits was checked visually on fully developed fruits before colour breaks.
2. Green stripes: Matured green fruits were visually observed and scored as either present or absent of green stripes.
3. Shape of fruit: Shape of ten fruits selected at random was observed visually and scored as flattened, slightly flattened, rounded, high rounded, heart-shaped, cylindrical and pyriform.
4. Fruit shape at blossom end: Ten ripe fruits were observed visually and the fruit shape at blossom end was scored as indented, flat and pointed.
5. Number of locules: Ten fruits were selected randomly and cut horizontally into two halves and the number of locules was counted.
6. Ribbing at peduncle end: Ten fruits were observed visually and the number of ribbings counted was recorded
7. Total soluble solids (^{o} Brix): Drop of extracted juice from two composite samples (made from 5 fruits each) was placed on the prism of a handheld refractometer and the average of the two readings was recorded.

4.2.5 Analysis of phenotypic data

The phenotypic data were analyzed using GenStat version 12th Edition. Four accessions out of the 123 accessions were not included in the analysis of the quantitative traits due to insufficient data. Multivariate analysis was also done using XLSTAT 2018.5.51780. Cluster analysis was performed using agglomerative hierarchical clustering based on Unweighted Pair-Group Average.

4.2.6 Analysis of molecular data

The SNP data from the 348 loci were analyzed and 10 SNP markers that were not informative were removed. The GenAlEx 6.501 programme was used for the estimation of the following parameters: observed and expected heterozygosities, polymorphic information content and principal coordinate analysis.

4.3 Results

4.3.1 Variability in qualitative traits

There was considerable variation among the genotypes for the qualitative traits studied (Table 4.1). Of the 123 accessions studied, 6% were determinate, 36 were semi-determinate and 58 were indeterminate. 11% of the germplasm evaluated had sparse foliage density, 34% had intermediate foliage density and 55% had dense foliage density. In terms of leaf coverage of fruits, 6% had exposed fruits, 39% had few leaves covering the fruits and 55% had their fruits hidden. A total of 67% of the accessions did not have green shoulders. All the accessions, with the exception of 1% did not have green stripes. Most of the fruits had pointed shape at blossom end (50%), followed by flat shape at blossom end (36%) and the least was indented at blossom end (14%). Majority of the accessions had intermediate fruit firmness (49%), followed by firm (37%) and soft (14%). The most common fruit shape recorded was flattened (30%), followed by highly rounded (28%) and slightly flattened (27%) - (Figure 4.1).

Table 4. 1: Distribution of qualitative traits of 123 tomato germplasm.

Traits	**Description and class**	**Frequency of class %**									
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
GT	1= Determinate; 2 =Indeterminate		42	58							
FD	3= Sparse; 5= Interminate; 7= Dense				11		34		55		
FGS	0= Absent; 1= Present	67	33								
LC	3= Sparse; 5= Intermediate; 7= Dense				6		34		55		
FGSt	1= Absent; 9= Present		99								1
SBE	1= Indent; 2= Flat; 3 =Pointed		36	14	50						
FF	3= Soft; 5= Intermediate; 7= Firm				14		49		37		
FS	1= Flattened; 2= slightly flattened; 3= Rounded; 4= High rounded; 5= Heart-shaped; 6= Cylindrical; 7= Pyriform; 8= Ellipsoid		30	27	3	28	2	6	2	2	

GT= Growth Type; FD= Foliage Density; LC= Leaf Coverage; FGS= Fruit Green Shoulders; FGSt= Fruit Green Stripes; SBE= Shape at Blossom end; FF= Fruit Firmness; FS= Fruit Shape.

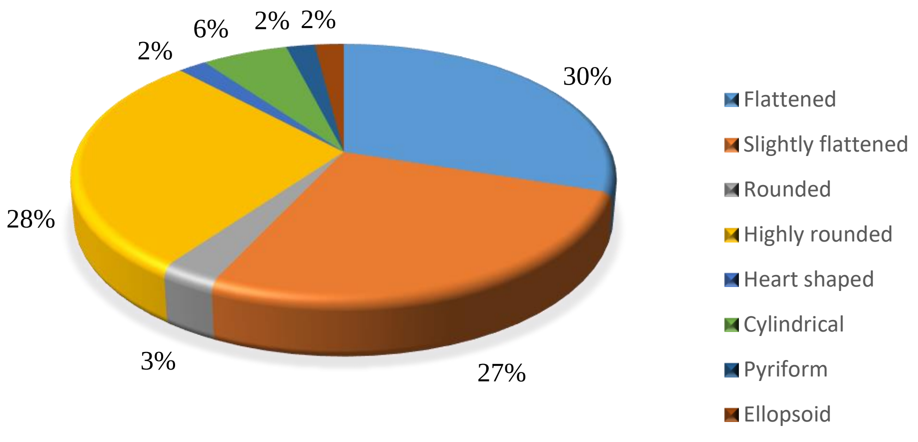

Figure 4. 1: Proportion of fruit shape distribution of 123 accessions evaluated

4.3.2 Genetic variation for vegetative, reproductive and fruit quality traits

There were significant differences ($p < 0.01$) among genotypes for number of days to first fruit set (NDFFS), days to maturity (TM), reproductive duration (RD) and number of fruits per plant (Table 4.2).

Table 4. 2: Mean square for various traits of 119 tomato genotypes

Sources of variation	**Mean Square**				
	Days to first fruit set	**Days to maturity**	**Reproductive duration**	**Fruits/plant**	**Brix**
Genotype	49.27**	142.96**	106.50***	2339.35***	0.61

4.3.3 Means of various traits studied for the 119 tomato accessions evaluated

Number of days to first fruit set (NDFFS) varied from 11 Days after Transplanting (DAT) in GH9185 to 67 DAT in genotype 08TEP080187 (Table 4.3). The NDFFS in GH9185 was significantly different from all the other genotypes. Power Rano produced fruits earlier (28 DAT) than Peto Mech (34 DAT). Days to maturity (TM) also varied from 49 DAT in F_1 Cobra 26 to 101 DAT for 10TEP080188. Genotypes Tom 1999 had the shortest reproductive duration

(18 DAT) and was not significantly different from F_1 Cobra 26 (19), GH9111 (I9), 08TEP070547 (19), BA-5 *Pimpinellifolium* x Wosowoso (20), LA3152 (22), and GH9208 (23) days.

Genotype LA2644 had the lowest fruit weight of 1.96 g/fruit whereas genotype Tomato oxheart had the heaviest fruit (240 g/fruit). The weight of Peto Mech (38 g/fruit) was significantly heavier than Power Rano (31 g/fruit). On the contrary, Power Rano recorded an average of 31 fruits/plant which was significantly many more fruits than Peto Mech that had an average of 22 fruits/plant. Genotype GH9078 had the highest number of fruits of 273 fruits/plant and was significantly different from all the 119 genotypes evaluated. In terms of yield, GH9310 gave the highest yield of 55.46 t/ha. Power Rano yielded 36.79 t/ha but was not significantly different from Peto Mech (32.2 t/ha). The expanded table is in the Appendix (Table S2).

Table 4. 3: Means of reproductive, yield component and fruit quality traits of 26 of 119 tomato germplasm.

Genotype	NDFFS (days)	TM (days)	RD (days)	FPP	Weight (g/fruit)	Yield (t/ha)	Brix
08TEP070547	50	69	19	12	52.51	25.32	3.95
08TEP080187	67	101	33	17	9.04	18.66	4.41
10TEP080188	32	101	68	56	4.14	27.65	5.61
14A112	31	62	31	2	50.8	28.17	4.60
15TEP070136	52	90	38	4	54.32	19.71	3.60
Pimpinellifolium x Woso	33	53	20	55	9.99	33.00	5.10
F_1 Cobra 26	30	49	19	19	49.66	39.70	2.90
F_1 Nadira	29	55	26	19	53.78	44.61	2.30
GH 9114 (Lorry Tyre)	35	62	27	44	30.24	41.33	3.25
GH9078	30	54	24	273	13.24	46.00	5.45
GH9107	32	60	28	83	17.91	39.52	4.95
GH9111	32	51	19	59	8.18	29.75	5.90
GH9185	11	60	49	50	8.12	20.36	4.50
GH9208	28	51	23	141	5.6	44.67	5.5
GH9233	25	52	27	215	3.25	36.23	5.90
GH9310	28	53	24	176	14.22	55.46	5.71
Heinz 1370	51	101	49	6	45.24	17.30	4.80
LA1793	33	93	60	27	17.86	19.63	4.05
LA1802	38	99	61	6	23.18	15.15	4.50
LA2644	31	64	33	113	1.96	25.39	6.45
LA3012	31	101	70	6	20.71	15.78	4.15
LA3152	34	56	22	7	51.03	24.53	4.50
LA4285	41	101	60	8	39.47	16.30	4.55
Larisa	20	58	38	4	73.26	24.03	3.50
Tom 1999	34	52	18	17	44.38	25.12	4.90
Tomato Oxheart	33	73	39	20	240.43	52.71	4.41
GH9193 (Power Rano)	28	56	28	31	30.51	36.79	4.49
Peto Mech	34	63	29	22	38.22	32.20	4.01

NDFFS=Number of days to first fruit set; TM=Time to fruit maturity: RD=Reproductive Duration; Fr/Pl=Fruits per plant

4.3.4 Principal Component Analysis

The principal component analysis (PCA) indicated that the first five principal components explained more than 80% of the phenotypic variation among the 119 genotypes with the first two components accounting for 53% of the variation (Table 4.4).

Table 4. 4: Eigenvalues and cumulative proportion due to thirteen phenotypic traits studied among 119 tomato genotypes

No.	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Eigenvalue	4.5	2.42	1.38	1.11	1
Proportion	0.35	0.19	0.11	0.09	0.08
Cumulative	0.35	0.53	0.64	0.72	0.8

The first principal component was dominated by fruit attributes including fruit shape, ribbing at peduncle end, fruit green shoulders (FS), number of locules (NL), growth type (GT), shape at blossom end number (SaBE), fruits per plant (FPP) and firmness. The second principal component was dominated by days to first maturity, reproductive duration and yield (Table 4.5)

Table 4. 5: Contribution of traits to total variation accounted for by the first five principal components following analyses of 119 tomato varieties in the field

Variables	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**
FS	**0.588**	0.102	0.031	0.018	0.060
Ribbing	**0.557**	0.027	0.233	0.001	0.000
FGS	**0.355**	0.001	0.158	0.001	0.214
NL	**0.501**	0.036	0.289	0.001	0.021
GT	**0.401**	0.010	0.070	0.027	0.024
SaBE	**0.760**	0.009	0.002	0.012	0.035
FPP	**0.445**	0.130	0.232	0.030	0.003
WPF	0.243	0.008	0.247	0.019	**0.381**
Yield	0.172	**0.386**	0.004	0.064	0.128
Firmness	**0.404**	0.111	0.004	0.005	0.001
DM	0.035	**0.820**	0.038	0.034	0.011
NDFFS	0.014	0.197	0.002	**0.728**	0.043
RD	0.022	**0.579**	0.073	0.173	0.076

Variables in bold correspond for each variable to factor for which the squared cosine is largest

4.3.5 Biplot Analysis

The distribution of 119 tomato accessions in space of the first two principal components from the biplot analysis based on 13 traits is shown in Figure 4.2. Based on the angles between the vector variables, fruit shape (FS), growth type (GT), shape at blossom end (SaBE), weight per fruit (WPF) and firmness were positively correlated. Ribbing (R) was positively correlated with fruit green shoulders (FGS), and number of locules (NL) whereas fruit per plant (FPP) and yield were also positively correlated. The number of days to first fruit set (NDFFS), reproductive duration (RD) and days to maturity (DM) were highly correlated. Fruit per plant (FPP) was negatively correlated with yield

Accessions were grouped into three clusters. The first cluster was made up of late maturing genotypes. These included 08TEP080187, 10TEP080188, LA4285, Tom Sonia, Heinz 1370, LA1793 and 15TEP070136. The second cluster was made up of local cultivars such as genotypes GH 9078, GH 9233, GH 9235. GH 9310 among others. They had the lowest fruit weight but produced the highest number of fruits. Genotypes GH 9185, GH 9239 GH 9128, GH 9163 and GH 9107 among others had the highest number of locules and the highest number of ribbings. The third cluster was made up of commercial and improved varieties. They were heavy and firm (Figure 4.2).

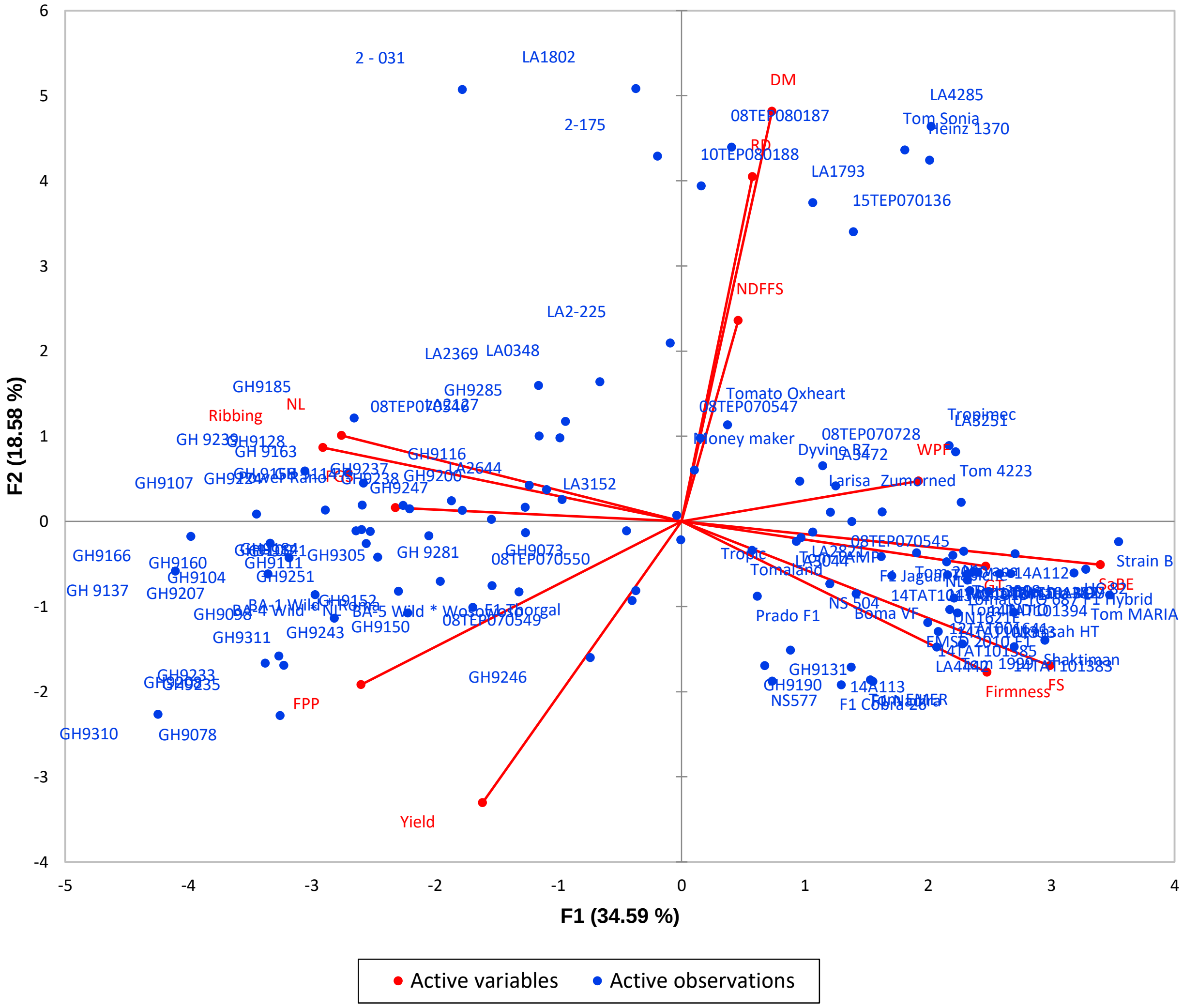

Figure 4. 2: Biplot analysis of thirteen traits of the 119 tomato accessions studied

4.3.6 Cluster Analysis

The hierarchical cluster analysis grouped the 119 tomato accessions into two clusters with Tomato oxheart as an outlier (Figure 4.3). Cluster I was made up of 81 genotypes (green colour), cluster II (blue) was made up of 37 genotypes. The variance within cluster II was about four times the variance in cluster 1(Table 4.6).

Table 4. 6: Class attributes obtained from the hierarchical cluster analyses among 119 tomato accessions based on thirteen traits in a field evaluation

Class	**1**	**2**	**3**
Genotypes	81	37	1
Sum of weights	81	37	1
Within-class variance	719.476	3035.482	0.000
Maximum distance to centroid	61.706	168.780	0.000

Genotypes in clusters I and II had about 54 % similarity. Genotype tomato oxheart was very high yielding, had the heaviest fruits, few fruits per plant, fruit green shoulders, many ribbings, many locules, heart-shaped, pointed shape at fruit blossom end, soft fruit and had determinate growth. Cluster II was mainly made up of the Ghanaian accessions which were largely characterized by medium to very high yielding, low fruit weight, many fruits per plant, many ribbings, many locules, flattened or slightly flattened fruit shapes, flat or indented shape at blossom end, mostly intermediate firmness, semi-determinate and indeterminate growth. Some of these genotypes had very high brix. Cluster I was made up of the commercial varieties, improved lines, introduced lines and a few local accessions. The three most widely grown accessions in Ghana were in cluster I. The commercial varieties were typically medium yielding, medium in weight, not too many fruit per plant, less ribbings, few locules, slightly flattened, rounded to high rounded fruit shape, flat or pointed shape at blossom end, intermediate to firm fruits with determinate or semi-determinate growth.

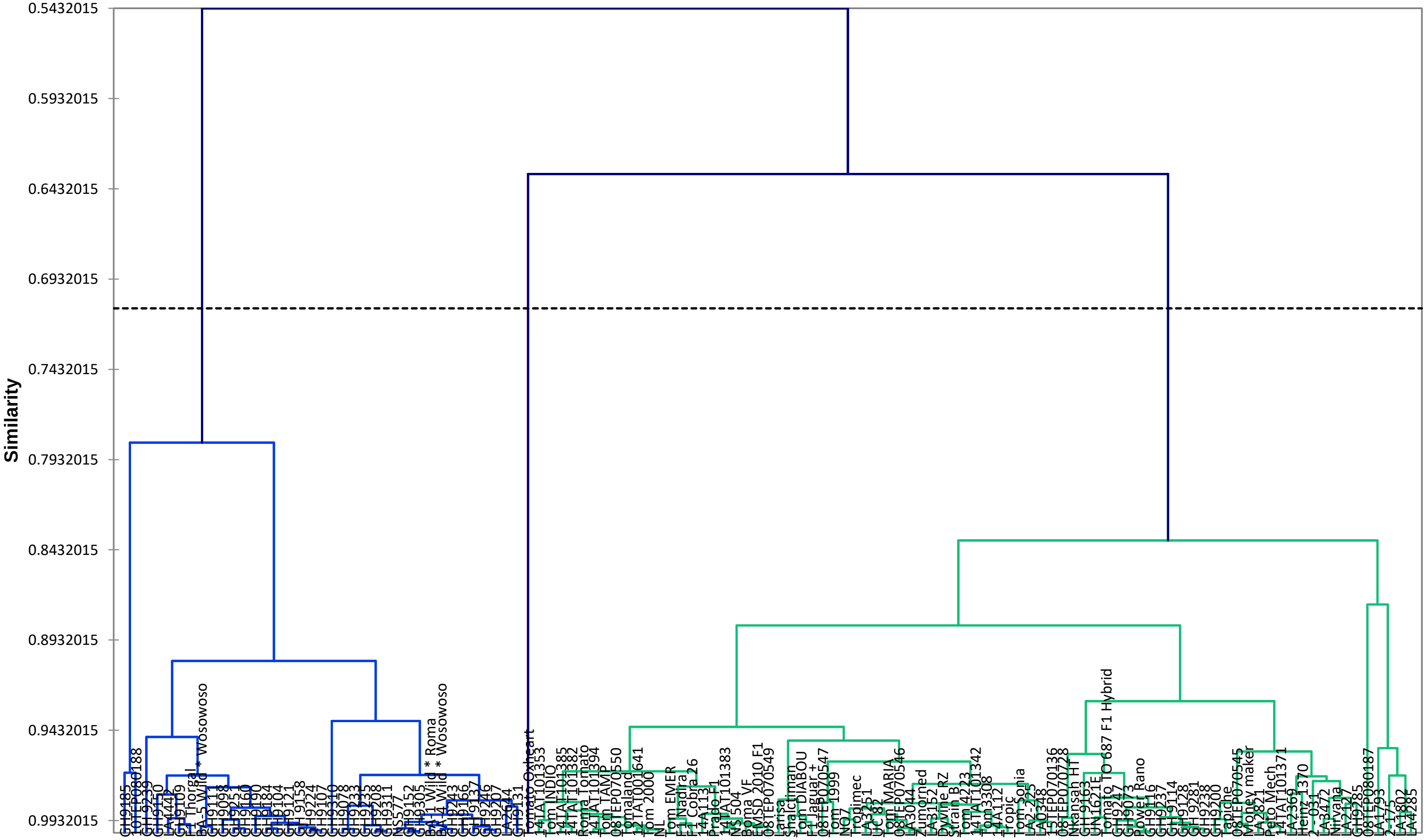

Figure 4. 3: Clustering based on reproductive and fruit quality traits of 119 tomato accessions

4.3.7 SNPs analysis of tomato accessions

Majority of the markers (97.04%) were polymorphic but 10 (2.96%) were monomorphic. A total of 330 of the 338 markers typed more than 90 individuals at a locus (Table 4.7). The observed homozygosity ranged from 0 to 0.94 with an average of 0.13 while the expected heterozygosity ranged from 0.06 to 0.50 with an average of 0.38. The Polymorphic Information Content (PIC) values also ranged from 0.06 to 0.38 with an average of 0.30. Of the 338 markers, 61% had a PIC of 0.30 to 0.38. The expanded table is in the Appendix (Table S4)

Table 4. 7: Range of key descriptive statistics for measuring the informativeness of 48 of the 338 SNPs markers based on 96 tomato accessions

Locus	N	Hobs	HExp	PIC	Locus	N	Hobs	HExp	PIC
S48097	82	0.00	0.25	0.22	S11092	90	0.13	0.46	0.35
S13458	95	0.00	0.06	0.06	S11205	96	0.15	0.39	0.31
S100154	96	0.10	0.49	0.37	S11205	96	0.15	0.39	0.31
S100197	94	0.16	0.28	0.24	S11231	95	0.20	0.50	0.37
S100205	96	0.10	0.49	0.37	S11281	95	0.06	0.19	0.17
S100240	96	0.08	0.36	0.29	S11543	95	0.21	0.46	0.35
S100246	93	0.08	0.09	0.09	S11588	96	0.06	0.10	0.09
S100269	96	0.16	0.35	0.29	S12200	93	0.16	0.50	0.37
S100516	96	0.16	0.50	0.37	S12201	96	0.20	0.44	0.34
S100561	96	0.22	0.48	0.36	S12212	94	0.12	0.49	0.37
S100691	96	0.07	0.44	0.34	S12213	96	0.12	0.49	0.37
S100743	96	0.16	0.50	0.37	S12372	96	0.16	0.42	0.33
S100810	94	0.04	0.17	0.16	S12414	94	0.14	0.43	0.34
S100981	96	0.03	0.18	0.16	S12421	91	0.11	0.25	0.22
S100987	96	0.12	0.29	0.24	S12501	95	0.05	0.09	0.09
S100995	95	0.17	0.46	0.35	S12535	96	0.06	0.12	0.11
S101009	96	0.05	0.18	0.16	S12536	96	0.06	0.12	0.11
S101067	96	0.08	0.40	0.32	S12638	96	0.17	0.48	0.36
S101068	95	0.08	0.27	0.23	S12647	96	0.23	0.49	0.37
S101085	96	0.12	0.23	0.20	S12656	94	0.12	0.29	0.25
S10372	96	0.14	0.34	0.28	S12664	95	0.02	0.08	0.08
S10686	96	0.13	0.29	0.25	S12718	96	0.17	0.44	0.34
S10796	96	0.07	0.20	0.18	S12749	95	0.28	0.44	0.34
S10958	96	0.13	0.33	0.28	S17645	96	0.94	0.0.50	0.38

N=Number of individuals typed at the locus, Hobs=Observed heterozygosity, Hexp=Expected heterozygosity, PIC=Polymorphic Information Content

Among the 96 accessions genotyped at 338 loci, the least number of heterozygous loci was 1 and the maximum number of heterozygous loci was 137 (Table 4.8). Of the 39 of local accessions genotyped, 12 of them had the lowest number of heterozygous loci values ranging from 1-10 (0.003-0.03 heterozygosity) and 9 genotypes had the highest number of heterozygous loci between 50 (0.15 heterozygosity) to 124 (0.37 heterozygosity). Of the 18 UC Davis accessions genotyped, 2 had the highest number of heterozygous loci values of 30 and 34 respectively. Five (5) out of 15 accessions received from FOHCREC had the lowest number of heterozygous loci between 1 (0.003 heterozygosity) and 3 (0.009 heterozygosity). All the accessions received from Wienco recorded high heterozygosity (0.10-0.40). The three most widely grown accessions (Peto Mech, Power Rano and GH9114) had heterozygous loci of 1, 2 and 3 respectively. The expanded table is in the Appendix (Table S5).

Table 4. 8: Level of heterozygosity of 82 of the 96 tomato accessions genotyped with 338 SNP markers

Sample	# Loci	# Het loci	Het	Sample	# Loci	# Het loci	Het
GH 9131	337	1	0.003	GH_9243	333	17	0.05
Nkansah HT	335	1	0.003	BA-5xWoso	333	18	0.05
Peto Mech	297	1	0.003	GH 9237	336	19	0.06
Money maker	338	2	0.006	GH 9235	335	19	0.06
LA3472	338	2	0.006	GH 9107	330	21	0.06
GH 9128	337	2	0.006	GH 9160	336	22	0.07
GH_9200	337	2	0.006	GH 9121	333	22	0.07
LA2-225	337	2	0.006	UC_82	333	26	0.08
Tomaland	337	2	0.006	GH 9305	334	27	0.08
LA1793	336	2	0.006	GH 9104	330	27	0.08
LA3012	335	2	0.006	Tropimec	334	28	0.08
Power Rano	335	2	0.006	LA3473	338	30	0.09
Boma VF	334	2	0.006	GH 9238	335	31	0.09
GH 9073	334	2	0.006	GH 9078	336	32	0.10
LA4440B	333	2	0.006	GH 9163	334	32	0.10
UN1621E	332	2	0.006	LA1018	337	34	0.10
Heinz 1370	337	3	0.009	GH_9247	333	34	0.10
LA2821	337	3	0.009	Roma tomato	310	32	0.10
GH 9114	336	3	0.009	BA-1xRoma	324	38	0.12
LA4442	336	3	0.009	GH 9109	333	40	0.12
LA3044	336	3	0.009	GH 9116	313	44	0.14
LA3152	336	3	0.009	GH 9207	331	50	0.15
LA2644	336	3	0.009	#15063	335	63	0.19
LA4440	335	3	0.009	GH 9285	331	65	0.20
GH 9184	335	3	0.009	NS577	330	65	0.20
LA2369	335	3	0.009	GH 9285	331	65	0.20
LA3151	334	3	0.009	NS577	330	65	0.20
LA2127	338	4	0.012	GH 9224	334	71	0.21
LA0348	338	4	0.012	GH_9152	332	71	0.21
GH 9281	334	4	0.012	GH_9098	331	71	0.22
GH 9233	331	4	0.012	Shaktiman	334	76	0.23
GH 9310	334	5	0.015	Nirvana	334	80	0.24
GH 9190	333	5	0.015	Dyvine_RZ	335	81	0.24
GH 9137	333	7	0.021	F$_1$_Nadira	328	89	0.27
Tomato Oxheart	333	9	0.027	Tom_Sonia	333	100	0.30
GH 9239	332	10	0.03	NO7	332	104	0.31
LA1802	336	11	0.033	Zumorned	335	108	0.32
GH 9158	333	14	0.042	F$_1$_Jaguar+	332	108	0.33
GH 9246	334	15	0.045	GH_9150	333	109	0.33
BA-4xWoso	337	17	0.05	Tom_TAMP	329	133	0.40
GH 9311	335	124	0.37	F$_1$ Thorgal	332	137	0.41

Loci=Number of Loci; #Het Loci=Number of heterozygous loci; Het=Heterozygosity

4.3.8 Principal Coordinate Analysis

Most of the variation was accounted for by variation among individuals within populations (52%) compared to variation within individuals (34%) or among populations (16%) though all three sources of variation were significant (Table 4.9). The first three principal coordinate axis explained more than 36% of the total variation (Table 4.10).

Table 4. 9: Mean square for 119 accessions based on 338 SNP markers**.**

Source	df	MS	Variation %
Among Populations	6	358.420***	16%
Among Individuals within populations	89	95.341***	52%
Within Individuals	96	22.396***	32%

Table 4. 10: Percentage of variation explained by the first 3 axes among 96 tomato accessions based on 338 SNP markers

Axis	PC 1	PC 2	PC 3
Percentage (%)	20.3	8.6	4.73
Cumulative Percentage (%)	20.3	28.9	33.62

Majority of the accessions from PGRRI were distributed in the first quadrant. Power Rano, Peto Mech and most of the commercial materials were in the second quadrant. Most of the accessions from UC Davis were scattered in the third and fourth quadrants (Figure 4.4).

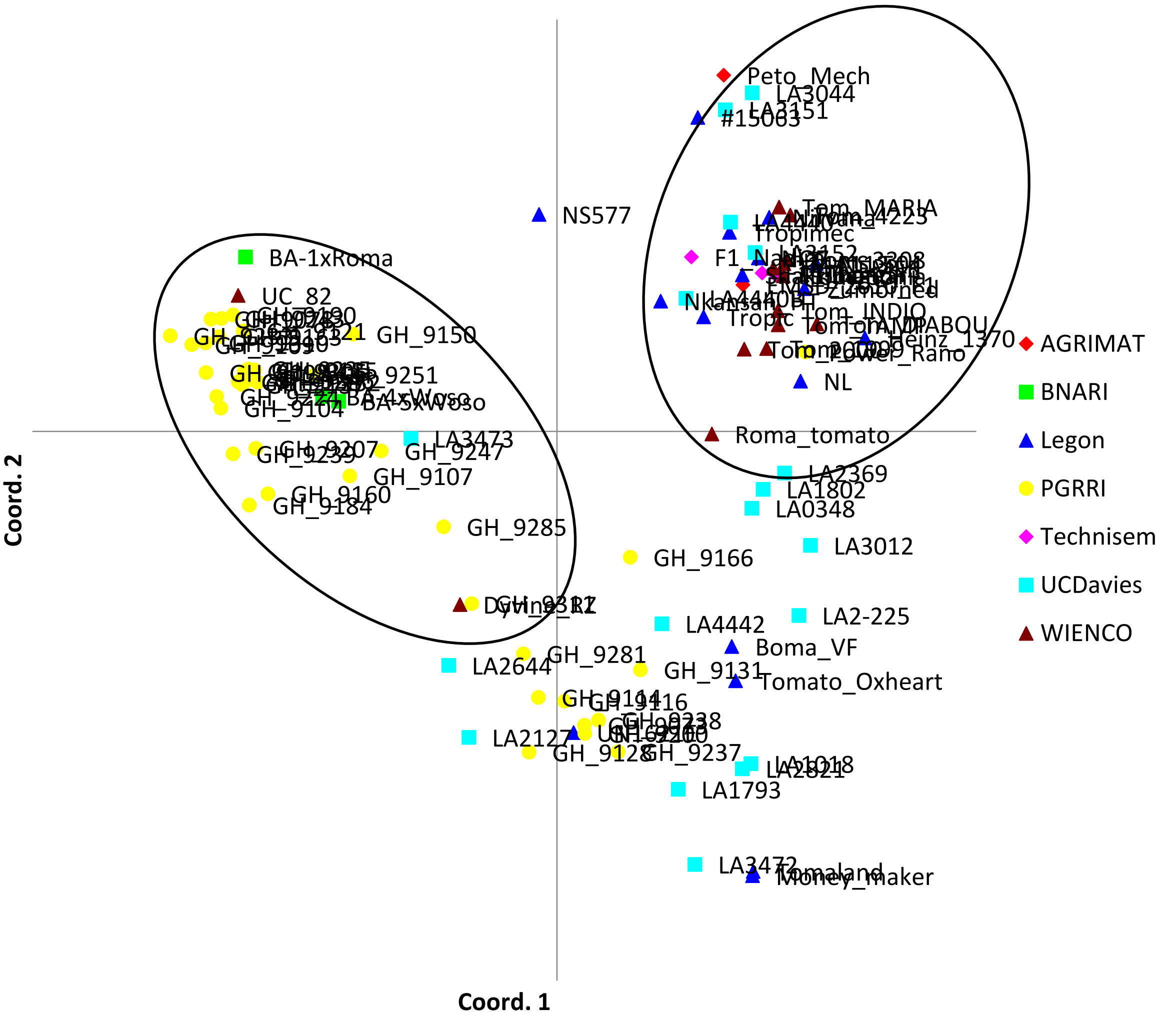

Figure 4. 4: Principal Coordinate Analysis of 96 tomato accessions based on 338 SNP markers

4.3.9 Association between SNP markers and shape at blossom end

Shape at blossom end appeared to have association with the SNP markers (Table 4.11). The PCoA showed that the first three axes explain 65.41% of total percentage variation for the association of fruit shape at blossom end with the SNP markers.

Table 4. 11: Percentage of variation explained by the first 3 principal components for association between 338 SNPs and fruit shape at blossom end

Axis	**PC 1**	**PC 2**	**PC 3**
Percentage (%)	51.5	9.1	4.81
Cumulative Percentage (%)	51.5	60.6	65.41

There was no association between the SNPs and all the traits studied with the exception of shape at blossom end. The association between the SNPs and fruit shape at blossom end grouped the accessions into II clusters. The first cluster was made up fruits with flat blossom end shape and the second cluster was made up of fruits with pointed blossom end shape (Figure 4.5).

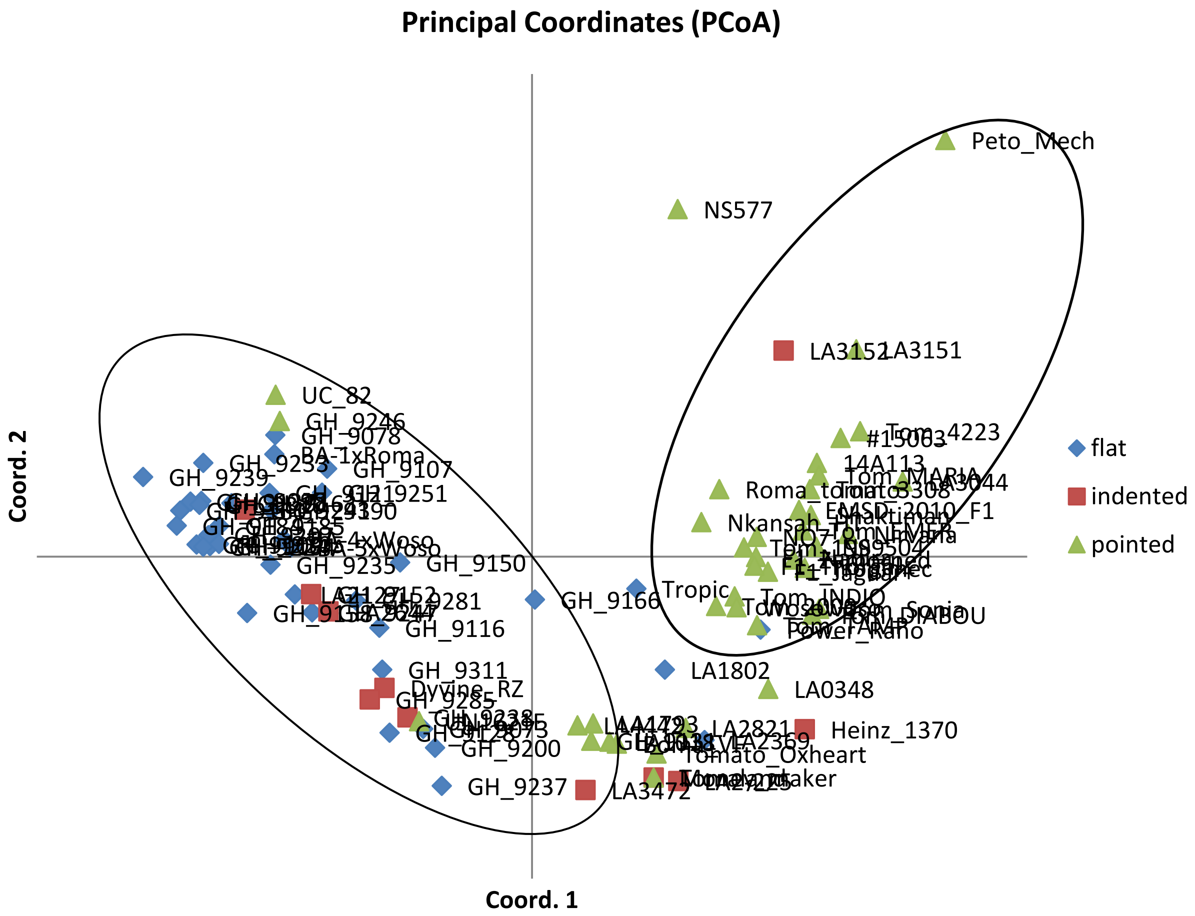

Figure 4. 5: Principal Coordinate Analysis for 96 tomato accessions based on association between 338 SNPs and Fruit shape at blossom end

4.4 Discussion

The success of a crop improvement programme depends on the extent of genetic variability existing in the germplasm. The extent of genetic variability can determine the pace and quantum of genetic improvement through selection or hybridization.

This study revealed the level of diversity within the tomato accessions based on morphological and molecular markers. There was significant variation among the accessions for the various traits studied and this agrees with the findings of Osei *et al*., (2014). The result is consistent with the findings of Kumar *et al*. (2013) who reported significant variation in days to maturity, number of fruits per plant and average fruit weight. Tembe *et al*. (2018) also reported significant variation in number of fruits per plant, fruit weight, fruit length and diameter.

It was found out that most of the local accessions had dense foliage density compared to the improved materials. The unimproved accessions were either semi-determinate or intermediate

Consumer and farming community preference for tomato traits vary from geographical area to the other throughout the world. Fruit shape, size, ribness and firmness are major traits consumers look out for in purchasing tomato. In this study, the predominant shape observed in the unimproved accessions was flat type and this agrees with the work done by Brewer *et al*. (2007) and Patil (2015). However, the improved varieties were typically rounded and high rounded and this agrees with the assertion that breeding techniques focused on developing rounded shape rather than flattened shape (NCARS, 1990). It was also observed that fruits that were flat or slightly flat were either soft or intermediate and ribbed compared to the high rounded shape fruits that were firm and smooth. Firmness of fruit plays an important role in the shelf life of tomato. Accessions that are pure lines based on the molecular analysis and were very firm can be exploited in developing fruits with long shelf life.

The unimproved accessions had very high brix compared to the improved accessions. The brix range of the accessions evaluated in this study agrees with the study of other authors who reported total soluble solids content ranged from 3.17 to 5.00 Brix (Shankar *et al*. 2013); 338 to 10.25 0 Brix (Reddy *et al*., 2013); 5.58 to7.53 0 Brix (Manna and Paul, 2012). Krishna *et al*. (2018) reported that breeding for bigger fruits dilutes the soluble sugar contents.

Yield is an important trait for both fresh market and processing, however, yield and yield component traits. The results of this study on variation in the weight per fruit and fruit yield agrees with various works such as 18-147 g (Chernet *et al*., 2013); 40.2-105.53 g (Shankar *et al*., 2013); 23-102.33 g (Reddy *et al*. 2013); 18-162 g and yield of 1.41-8.24 kg/plant (Patil, 2015)

Fruit per plant had negative correlation with weight per fruit but positively correlated with yield. This is because accessions with many fruits had smaller fruit sizes and were high yielding. This is is consistent with the findings of Tembe *et al*. (2018). However, Henareh *et al*. (2015) reported negative significant correlation between number of fruits per plant and yield. It was attributed to the large number of cherry tomato in their collection.

The 119 tomato germplasm studied were grouped into two distinct clusters. The outlier genotype (Tomato Oxheart) was made of one unique genotype that was high yielding, heavy and heart shaped. The accession can be utilized in improving fruit size. Cluster II was made up of genotypes that were not appealing to the fresh market but might be utilized for improving other traits particularly earliness. Genotypes in cluster I was largely made up of improved materials including the two most preferred varieties in Ghana. The few unimproved accessions found in this cluster can be utilized in a breeding programme for the fresh market. It was surprising to find the two most preferred varieties in Ghana among cluster II. This was intriguing because Power Rano was local accession and Peto Mech was an imported variety.

The number of heterozygous loci also had wide value range. The expected heterozygosity and polymorphic information content are used to measure the genetic diversity (Sim *et al*., 2012). From the results, few SNPs loci had low PIC values, which indicates a low allelic variation in the marker loci and their distribution among the tomato accession. Many loci (61%) showed PIC values of 0.30 to 0.38. PIC of 3 indicates moderate informativeness of the SNP markers used (Mateescum *et al*., 2005). Corrado *et al*. (2013) reported 30% of 175 SNPs had PIC of 0.30 to 30.37 in 214 tomato accessions. Cortes *et al*. (2011) reported PIC of < 0.20 and maximum value of 0.500 in common bean. Majority of the genotypes he reported had PIC of 0.40. The observed heterozygosity had a wider range compared to the expected heterozygosity. Observed heterozygosity was as high as 0.94. The first two principal components in this study (20.3% and 8.6%) were lower than 22% and 16% reported by Sim *et al*. (2012), using 7,720 SNPs (SolCAP SNP array) on a collection of 426 tomato accessions. The first three principal components are the most important in reflecting the variation with accessions (Adebisi *et al*., 2012) and therefore since the variation of the principal components was higher than 25%, then the information can be utilized together with cluster analysis to identify related genotypes (Ahmad *et al*., 2015). There were larger populations overlap for both coordinates, though major grouping can be seen for PGRRI, UC Davies and improved accessions.

It was realized that most of the accessions except those from IPRRI (local cultivars) and UC Davis had a high level of heterozygousity. The three most cultivated accessions tomato in Ghana, namely; Peto Mech, Power Rano and Lorry had low heterozygous loci and were widely separated in the PCoA. The genetic distance among tomato accessions gives an indication of the genetic relatedness.

4.5 Conclusion

The accessions were grouped into two clusters. Most of the Ghanaian accessions were in cluster II and had many fruits, ribbing at peduncle and locules. The fruits were also soft; making them unsuitable for fresh market. Based on both morphological traits and molecular markers the widely grown accessions; Peto Mech, Power Rano and GH9114 were in the same cluster as the improved varieties. The SNPs markers used distinguished pure lines and heterozygous lines as well as established the genetic similarity among accessions. Peto Mech and Power Rano were in the same cluster. Accessions from UC Davis area valuable addition to diversity in the accessions from PGRRI. Pure lines with good fruit quality traits can be utilized in future breeding programmes.

CHAPTER FIVE

5.0 Screening of tomato accessions for TYLCD resistance and identification of resistance genes

5.1 Introduction

Tomato production in Ghana is confronted with many constraints particularly pest and diseases. Among the diseases of tomato in Ghana, Tomato Yellow Leaf Curl Disease (TYLCD) is the most devastating which results in low or no yield (Osei *et al*., 2012).

Considering the worldwide importance of TYLCD, much effort has been devoted to its control. Earlier TYLCD prevention techniques focused on physical and chemical barriers that prevent whiteflies access to the plants. Recently, cultural practices aiming to reduce the viral inoculum load have led to some significant success (Ucko *et al*., 1998; Salati *et al*, 2002). However, the most viable option today is breeding varieties that are resistant to the virus or the whitefly vector (Polston and Lapidot, 2007). Six TYLCD resistance genes; *Ty-1/Ty-3, Ty-2, Ty-3, Ty-4, ty-5, Ty-6* have been mapped from wild tomato species and introgressed into cultivated tomato (Ji *et al*., 2007 a, b, c; Verlaan *et al*., 2013; Hutton and Scott, 2015; Hanson *et al*., 2016).

Even though various management techniques and resistant cultivars have been developed and deployed worldwide, TYLCD is still devastating in West Africa because of delayed adoption of effective TYLCD control techniques leading to very low yields and increased tomato imports.

In Ghana, most tomato research works have focused on screening exotic and local germplasm for resistance or susceptibility to the TYLCD (Osei *et al*., 200; Ossom 2012; Asare-Bediako *et al*., 2017; Segbefia *et al*., 2018). However, little effort has been made on identifying the known genes that confer resistance to the local TYLCV strains in Ghana and subsequently introgressing these genes into locally adapted lines.

Hence, the objective of this work was to identify TYLCD resistant materials and to introgress known TYLCD resistance genes into locally adapted cultivars.

5.2 Materials and Methods

5.2.1 Screening for known Ty gene Loci in the assembled germplasm

Primers for known TYLCD resistance genes (Table 5.1) were used to confirm or amplify new resistance genes in tomato accessions. The accessions used for the study were 21 as listed in Table 5.2. Some accessions were selected based on reported level of tolerance to TYLCD. Some accessions had different combinations of TYLCD resistance genes while some were selected based on their wide cultivation in Ghana. Other accessions were dropped because of insufficient seeds. The experiment was carried out at the Biotechnology Centre, University of Ghana.

Table 5. 1: List of primers used in the amplification of TYLCD resistance genes

Ty gene	**Marker name**	**Sequence**	**PCR product (bp)**
Ty-2	T0302	F: TGGCTCATCCTGAAGCTGATAGCGC R: AGTGTACATCCTTGCCATTGACT	Resistant: 900 Susceptible: 800
Ty-2	P1-16	F: CACACATATCCTCTATCCTATTAGCTG R: CGGAGCTGAATTGTATAAACACG	Resistant: 300 Susceptible: 600
Ty-3	P6-25	F: GGT AGT GGA AAT GAT GCTGCTC R: GCT CTG CCT ATT GTC CCA TAT ATA ACC	Resistant: 450, 650 Susceptible: 320
ty-5	TM273	F: GGTGCTCATGGATAGCTTAC R: CTATATAGGCGATAGCACCAC	Resistant:175 Susceptible 170
Ty-6	SLM10-46	F: TCGAGCTGGTACATAGCTTCAT R: CATCTGACACTTGGTCCAGAA	Resistant: 255 Susceptible: 220

5.2.1.1 DNA Extraction

Fresh leaf samples (200 mg) were harvested from the tomato genotypes at 14 days after germination and DNA was extracted using the CTAB method (Lodhi *et al.*, 1994). The quality of the DNA was determined by running the extracted DNA on 1% agarose gel stained with ethidium bromide. The DNA was stored in −20° C for later use.

Table 5. 2: List of Accessions used for detection of known TYLCD resistance genes and field screening in Ashanti and Upper East Regions

	Accession	**Code**	**Reported TYLCD resistance genes**	**Source**
1	AVTO1219	D1	*Ty-1/Ty-3, Ty-2*	AVRDC
2	AVTO1311	D2	*Ty-1/Ty-3*	AVRDC
3	AVTO1429	D4	*Ty-2, Ty-3*	AVRDC
4	AVTO1424	D5	*Ty-2, Ty-3*	AVRDC
5	AVTO9802	D6	*Ty-1, Ty-2*	AVRDC
6	AVTO0301	D8	*Ty-2*	AVRDC
7	AVTO1350	D9	*Ty-1, Ty-2*	AVRDC
8	GH9233 (Pimplifolium)	G5	unknown	PGRRI
9	*Pimpinellifolium* x Wosowoso	G121	unknown	BNARI
10	LA4440a	G153	unknown	UC Davis
11	GH 9193 (Power Rano)	C-1	unknown	PGRRI, Bunso
12	GH 9114 (Lorry Tyre)	G9	unknown	PGGRI, Bunso
13	Peto Mech	C-2	unknown	Agrimat
14	Nadira	N	unknown	Technisem
15	Tomato T0 687 F_1 Hybrid	G47	unknown	Syngenta
16	Tapiche	G48	unknown	Syngenta
17	P005A	A	unknown	Farmer
18	P005B	B	unknown	Farmer
19	Thorgal	TH	unknown	Technisem
20	Wosowoso	W	unknown	Crop Science
21	Power	R	unknown	Farmers

5.2.1.2 Polymerase Chain Reaction (PCR)

A total of 25µl reaction mix was used for the amplification of the known TYLCD resistance genes. The reaction mix contained 1x OneTaq, 2_X Master Mix with Standard Buffer, 0.2µM of forward and reverse primers and 100 ng of DNA. The amplification was done in a pre-heated Applied Biosystems 2720 Thermal Cycler with different conditions for each primer (Table 5.3).

The PCR products were separated on 1.5% agarose gel stained with ethidium bromide and visualized under UV light.

Table 5. 3: PCR conditions for primers used in the amplification of TYLCD resistance genes.

P1-16 (Hanson, 2016)		P6-25 (Ji *et al*., 2010)		TM273 (Hanson, 2016)	
Temp. (°C)	**Time(min)**	**Temp.(°C)**	**Time(min)**	**Temp.(°C)**	**Time(min)**
1. 95	10:00	1. 94	04:00	1. 95	5:00
2. 94	0:30 (34x)	2. 94	00:30 (35x)	2. 94	0:30 (34x)
3. 55	0:45	3. 53	1:00	3. 55	0:30
4. 72	0:45	4. 72	1:00	4. 72	0:30
5. 72	5:00	5. 72	10:00	5. 72	5:00
6. 20	∞			6. 20	∞
SLM10-60, (Hanson, 2016)					
Temp. (°C)	**Time(min)**				
1. 95	5:00				
2. 94	0:30 (34x)				
3. 58	0:30				
4. 72	0:30				
5. 72	5:00				
6. 20	∞				

5.2.2 Field screening of tomato accessions against TYLCD in Akumadan and Vea and molecular confirmation of viral DNA in infected Plants

A total of 21 tomato accessions (Table 5.2) were screened for their reaction to TYLCV in two disease hotspots at the Dam site in Akumadan (Ashanti Region) and Vea Irrigation Site (Upper East Region) to ascertain the resistance of the tomato accessions to the local strains of TYLCV present in the areas.

5.2.2.1 Experimental design

Both field experiments were laid out as Randomized Complete Block Design (RCBD) with three (3) replications. Every genotype was planted in two rows of 10 plants per row. The rows were 1m apart. Plants within a row were 0.5 m apart and the row length was 4.5 m.

5.2.2.2 Agronomic practices

Tomato seeds of each accession were sowed in nursery trays. Thirty-day-old seedlings were transplanted to the field. Watering was done when necessary. At two weeks after transplanting, N.P.K. 15-151-15 was applied at the rate of 10g per plant. At four weeks after transplanting, Sulphate of Ammonia was applied at the rate of 5g per plant. Calcium nitrate (Fertigation grade) was applied at fruiting at the rate of 150 g/L. Fruit borers were controlled with Dipel at the rate of 100 g/100 L of water.

5.2.2.3 Molecular confirmation of viral DNA in infected leaf samples

Fresh leaves of infected plants of 21 tomato accessions were sampled at 60 days after transplanting (DAT) from both locations and stored on ice and brought to the Biotechnology Laboratory, University of Ghana for DNA extraction using CTAB method.

5.2.2.4 Polymerase Chain Reaction

A total of 25µl reaction mix was used for the amplification of the viral DNA in the infected samples. The reaction mix contained 1x OneTaq, 2$_X$ Master Mix with Standard Buffer, 0.2µM of forward and reverse primers and less than 100 ng of DNA. Three (3) degenerate and two (2) specific primers (Table 5.4) were used. PCR conditions used for all the five primers were; 94°

C for 1 minute, 94° C for 1 min (30 cycles), annealing temperature of 53° C for 1 min, 72° C for 1 min, final extension of 72° C for 10 min and held at 4° C infinity. The PCR products were separated on 1.5% agarose gel stained with ethidium bromide and visualized under UV light.

Table 5. 4: Primers used in the amplification of TYLCV in infected samples

Marker Name	Primer sequence	References
PARc1496/PAL1v1978	F:5'GCATCTGCAGGCCCACATYGTCTTYCCNGT R: 5'AATACTGCAGGGCTTCTRTACATRGG	Rojas *et al*. (1993)
AV494/AC1048	F: GCCCATGTATAGAAAGCCAAG R: GGATTAGAGGCATGTGTACATG	Wyatt and Brown (1996)
PTYv787/PTYc 1121	F: 5-GTTCGATAATGAGCCCAG-3 R: 5-ATGTAACAGAAACTCATG-3	Zhou *et al*. (2008)
GHF/GHR	F: GCCCGAAAGCTTCGTTGTT TTCCCGCT R: ACGGATGGCCGCTTTGGGT ATTCG	Osei *et al*. (2008
KF/KR	F: GGACCCGGCGCACTATTTAT GTTGGC R: ACCCCATTACCCCAATACCA	Osei *et al*. (2008

5.2.2.5. Visual Scoring of TYLCD symptoms at Akumadan and Vea

The incidence and severity of the disease was scored at 30, 45 and 60 days after transplanting (Lapidot and Friedman, 2002) using the scale 1–6 severity, where: 1 = healthy, no observable symptoms; 2 = very mild with slight yellowing and mosaic on top leaves and no leaf curling; 3 = mild yellowing, mosaic and/or slight leaf curling on youngest leaves, severe symptoms; 4 = moderate yellowing and/or leaf curling on the youngest (top) leaves; 5 = severe yellowing and blistering and/or severe leaf curling plus some leaf size reduction on the youngest leaves of the main stem and/or at least one branch; 6 = very severe yellowing, blistering and/or very severe leaf curling, leaf deformation, leaf size reduction and stunting (Hanson *et al*., 2016). The TYLCD incidence was calculated as follows (Imran *et al*., 2012);

$$\text{Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \text{ x } 100$$

5.2.2.6 Yield and Yield Component Data

Data were also collected on number of fruits per plant, average fruit weight, yield, fruit diameter and fruit length.

5.2.3 Data Analysis

Phenotypic Analysis

GenStat statistical package Edition 12.1 was used to analyze the quantitative data. Means were separated by Fisher's protected Least Significant Difference (LSD) at 95% confidence interval.

5.3 Results

5.3.1 Amplification of known TYLCD resistance genes in tomato accessions

The reliability and diagnostic capabilities of five molecular markers previously reported to be linked to TYLCD resistance genes were evaluated and validated for the purpose of introgression.

5.3.1.1 Marker Analysis

The gels for primer T0302 showed susceptible band in most of the samples. For the other primers, only gels showing at least one resistant band for the 21 accessions were presented amplified at least one The *Ty-2* linked marker (T0302 F/R) produced susceptible fragment of approximately 800 bp in accessions C-1 (Power Rano), BG9, G5 (Pimplifolium), G153 (LA4440a), R (Power), G9 (Lorry Tyre), AVTO1311, P005B, Tapiche, Thorgal and LT (Plate

5.1). Another Ty 2 linked marker P1-16 F/R (*Ty-2*), amplified resistant fragment of approximately 300 bp in accessions D1 (AVTO1219) and D4 (AVTO1429) (Plate 5.2). *Ty-3* linked marker P6-25 amplified resistant band (450 bp) in genotypes D1 (AVTO1219), D2 (AVTO1311), D4 (AVTO1429) and (G48) Tapiche (Plate 5.3). The *ty-5* primer TM273 amplified about 230 bp in genotype G5 (Pimplifolium) (Plate 5.4). The *Ty-6* primer SLM10-46 amplified a band of approximately 255 bp in genotypes G5 (Pimplifolium) and G121 (*Pimpinellifolium* x Wosowoso) (Plate 5.5).

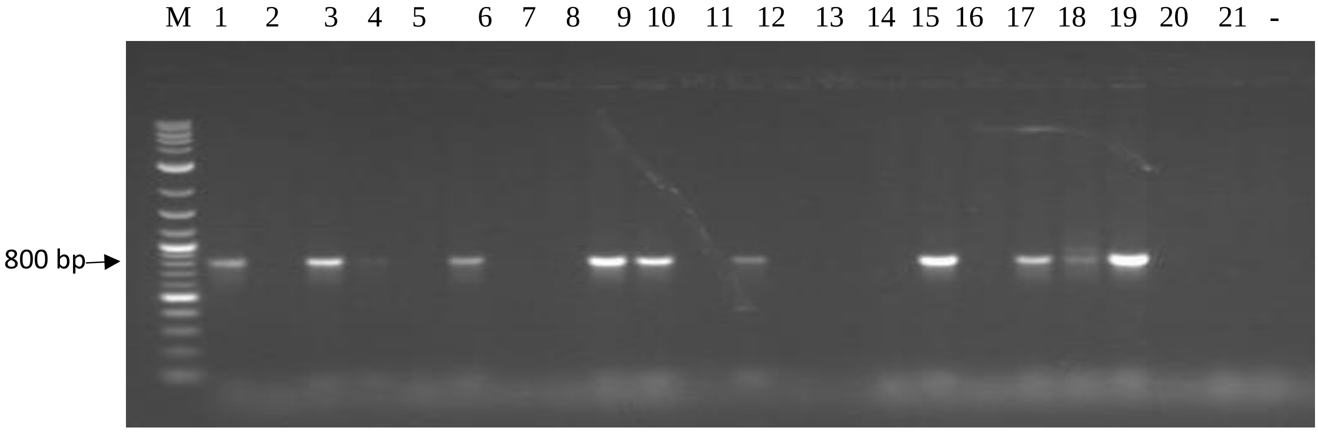

Plate 5. 1: PCR amplification products of *Ty-2* gene obtained from 21 accessions of tomato germplasm using T0302 F/R primer pair. Lanes: 1= C-1, 2=C-2, 3=BG9, 4=G5, 5=W, 6=G153, 7=N, 8=G121, 9=R, 10=G9, 11= D1, 12=D2, 13=D3, 14=D4, 15=A, 16=B, 17=S1, 18=S2, 19=TH, 20=LT, 21=D5; M=2- Log DNA ladder; -Ve = Negative control (sterile nuclease free water) . Susceptible: 800 bp; Resistant: 900 bp

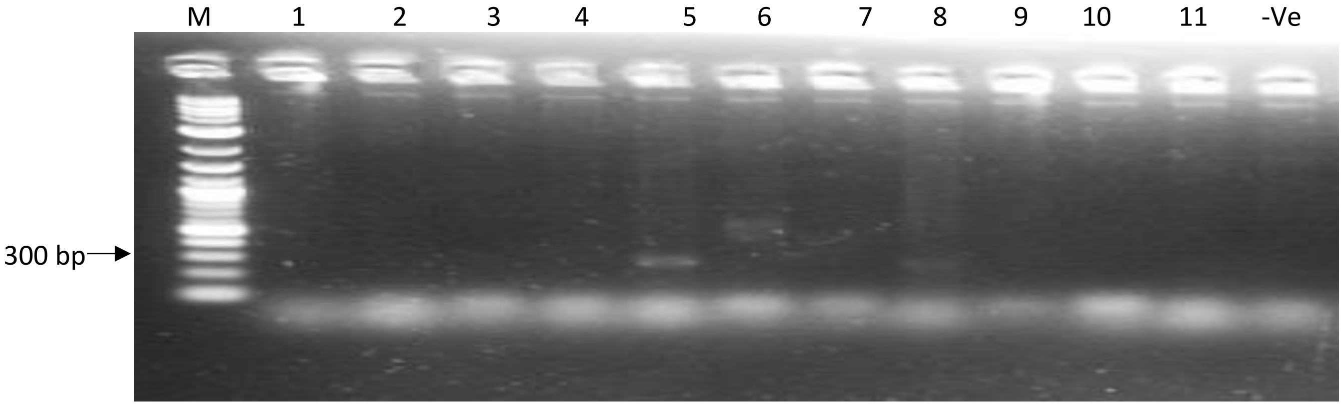

Plate 5. 2: PCR amplification products of *Ty-2* gene obtained from 11 accessions of tomato germplasm using P1-16 F/R primer pair. Lanes: 1= A1, 2=A2, 3=A3, 4=A4, 5=D1, 6=D2, 7=D3, 8=D4, 9=G5, 10=G121, 11= 153; -ve = Negative control (sterile nuclease free water); M=2- Log DNA ladder. Susceptible Band: 600 bp; Resistant 300 bp

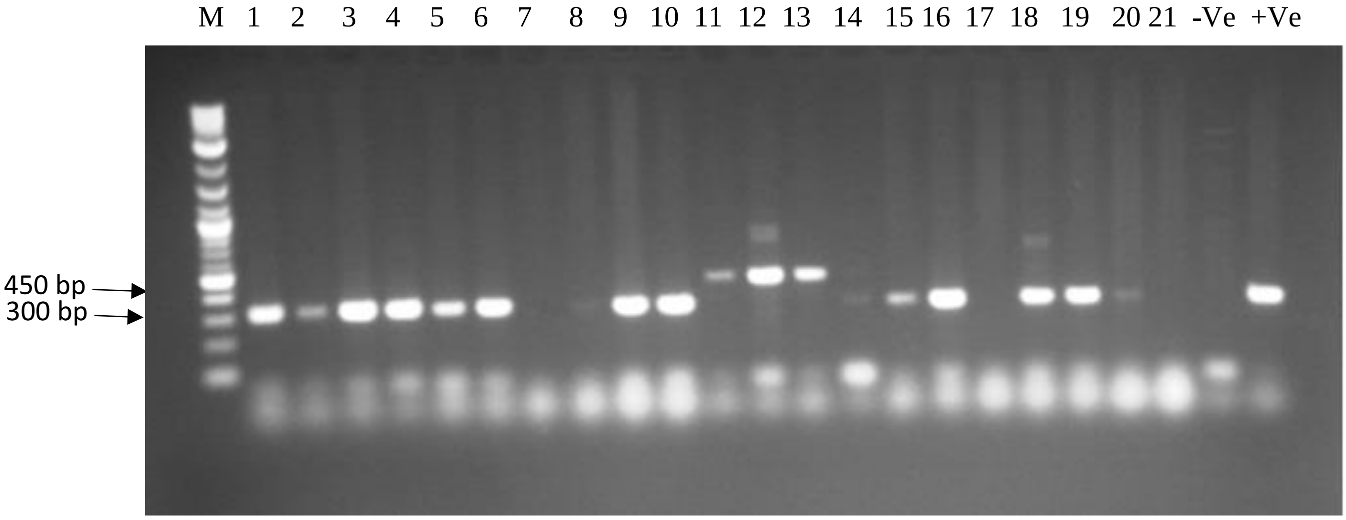

Plate 5. 3: PCR amplification products of *Ty-3* gene obtained from 21 accessions of tomato germplasm using P6-25F/R primer pair. Lanes: 1= C-1, 2=C-2, 3=BG9, 4=G5, 5=W, 6=G153, 7=N, 8=G121, 9=R, 10=G9, 11= D1, 12=D2, 13=D4, 14=D3, 15=A, 16=B, 17=S1, 18=S2, 19=TH, 20=LT, 21=D5; M=2-Log DNA ladder; -Ve = Negative control (sterile nuclease free water), +Ve = Positive control (B);. Susceptible Band: 320 bp; Resistant: 450 bp

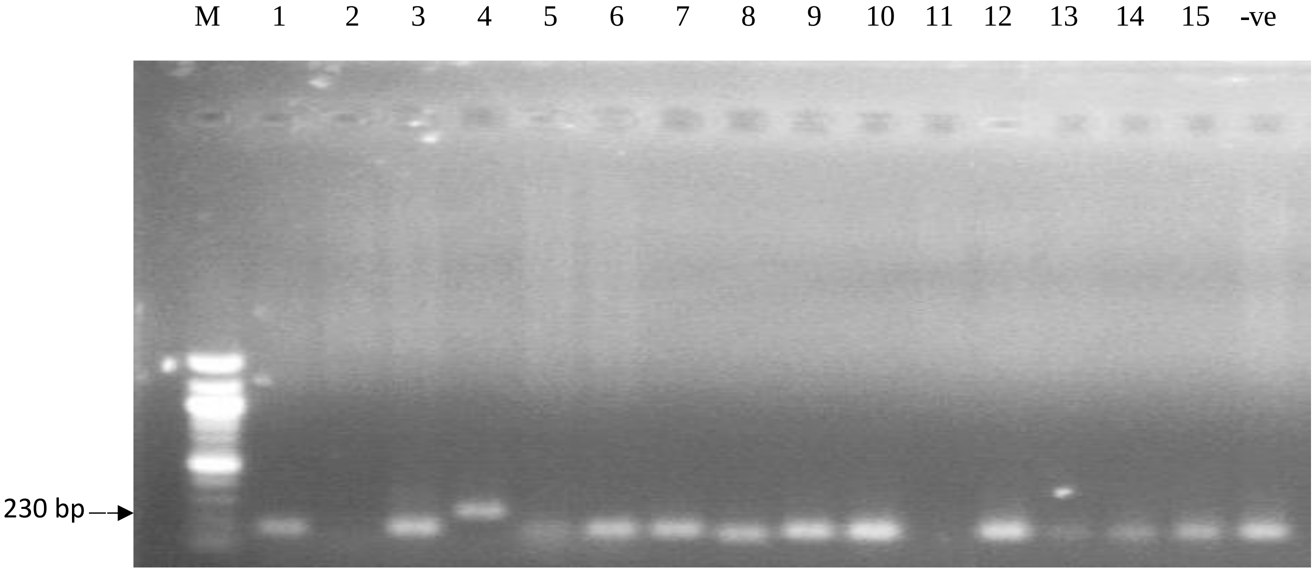

Plate 5. 4: PCR amplification products of *ty-5* gene obtained from 16 accessions of tomato germplasm using F/R primer pair. Lanes: 1= C-1, 2=C-2, 3=G9, 4=G5, 5=W, 6=G153, 7=LA3473 P1, 8=LA3473 P2, 9=L14440 P1, 10=GLA14440 P2, 11= D1, 12=D2, 13=D3, 14=D4, 15=A; M=2- Log DNA ladder; -Ve = Negative control (sterile nuclease free water). Susceptible: 170 bp; Resistant: 175 bp

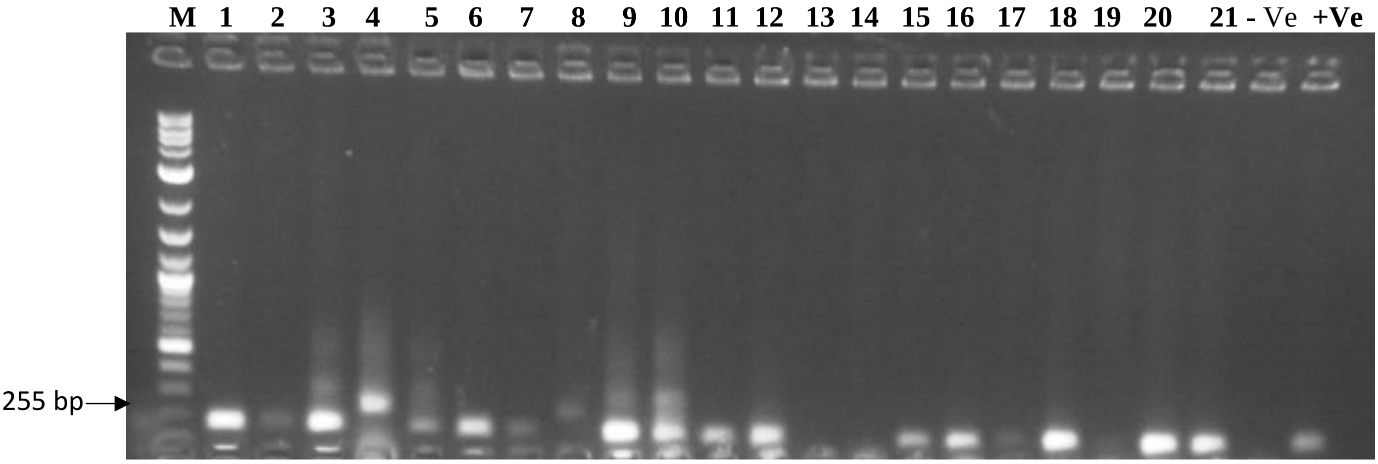

Plate 5. 5: PCR amplification products of *Ty-6* gene obtained from 21 accessions of tomato germplasm using SLM10-46 F/R primer pair. Lanes: 1= C-1, 2=C-2, 3=BG9, 4=G5, 5=W, 6=G153, 7=N, 8=G121, 9=R, 10=G9, 11= D1, 12=D2, 13=D4, 14=D3, 15=A, 16=B, 17=S1, 18=S2, 19=TH, 20=LT, 21=D5; M=2- Log DNA ladder; -Ve = Negative control (sterile nuclease free water); +Ve = Positive control (B). Susceptible: 220 bp; Resistant: 255 bp

5.3.2 Incidence and Severity of TYLCD resistance in Akumadan and Vea Irrigation site

5.3.2.1 Disease incidence ratings

Variation in disease incidence was significant (5%) at all the various stages of growth at both locations at 30 DAT, 45 DAT and 60 DAT.

All the accessions screened against the local strain of TYLCV in Akumadan expressed symptoms by 60 DAT (Table 5.5). On the contrary, not all the accessions expressed TYLCD symptoms by 60 DAT at Vea irrigation site. The TYLCD incidence ranged from 1.67% to 60%, 13.33% to 91.17% and 44.68% to 100% at 30 DAT, 45 DAT and 60 DAT respectively in Akumadan. At 60 DAT genotype Pimplifolium had the lowest incidence (44.68%) while genotypes AVTO1311, AVTO1424, AVTO030, Lorry Tyre, Peto Mech and Tomato T0 687 F_1 Hybrid had the highest incidence (100%).

The TYLCD incidence at Vea ranged from 0% to 20.86%, 0% to 39.2% and 0% to 84.17% at 30 DAT, 45 DAT and 60 DAT respectively. Pimplifolium recorded no symptoms throughout the experiment (Table 5.5).

Table 5. 5: Incidence of TYLCD on plants of 21 tomato accessions at 30 DATS, 45 DAT and 60 DAT at Akumadan and Vea

Akumadan				**Vea**			
Genotype	**30 DAT**	**45 DAT**	**60 DAT**	**Genotypes**	**30 DAT**	**45 DAT**	**60 DAT**
Peto Mech	51.33	72.20	100.00	Peto Mech	20.86	39.27	84.17
AVTO1311	44.00	91.17	100.00	Thorgal	12.17	27.57	66.78
AVTO1424	60.00	88.93	100.00	Lorry Tyre	17.2	30.87	62.30
AVTO0301	58.33	77.23	100.00	P005B	12.26	22.14	61.43
Tomato T0 687 F_1 Hybrid	56.67	86.90	100.00	AVTO9802	1.75	9.45	60.81
Lorry Tyre	47.33	69.03	100.00	Tomato T0 687 F_1 Hybrid	0.00	11.60	59.19
AVTO1429	29.33	72.67	97.90	AVTO0301	17.55	17.55	58.46
AVTO1219	54.00	86.67	97.60	AVTO1424	3.33	5.73	56.93
Tapiche	53.67	73.8	96.97	Nadira	1.85	5.92	52.42
AVTO1350	35.33	54.00	96.67	LA4440a	0.00	20.20	51.50
AVTO9802	42.67	69.60	96.30	Tapiche	6.67	20.9	47.17
LA4440a	25.67	65.37	96.30	AVTO1350	1.85	17.67	47.14
P005A	38.33	72.63	95.57	*Pimpinellifolium* x Wosowoso	0.00	19.60	43.63
Thorgal	41.33	71.00	95.23	Power	12.2	38.33	41.64
Power	28.67	52.70	89.97	AVTO1311	0.00	3.72	37.8
Nadira	42.00	50.80	89.93	Power Rano	14.07	22.17	27.40
P005B	35.00	72.63	88.80	P005A	9.27	14.76	26.67
Power Rano	19.67	60.70	86.77	AVTO1219	2.38	2.38	21.57
Wosowoso	10.43	83.00	86.37	AVTO1429	0.00	2.08	18.09
Pimpinellifolium x Wosowoso	13.00	29.97	74.63	Wosowoso	5.88	6.67	5.59
Pimplifolium	1.67	13.33	44.68	Pimplifolium	0.00	0.00	0.00
Mean	37.50	66.20	92.10	Mean	6.63	16.10	44.3
L.s.d (5%)	29.20	25.08	20.01	L.s.d	11.489	13.18	21.18

5.3.2.2 Disease severity rating

Generally, all the genotypes expressed very severe symptoms in Akumadan than Vea. At Akumadan, severity values ranged from 1 to 4, 2 to 5 and 2 to 5 at 30 DAT, 45 DAT and 60 DAT respectively (Tables 5.6). At Vea, TYLCD severity ranged from 1 to 3, 1 to 4 and 1 to 4 at 30 DAT, 45 DAT and 60 DAT respectively.

Pimplifolium expressed very mild with slight yellowing and mosaic on top leaves and no leaf curling at 60 DAT at Akumadan. P005B expressed moderate yellowing and/or leaf curling on the youngest (top) leaves while P005A, Power Rano and Peto Mech expressed severe yellowing and blistering and/or severe leaf curling plus some leaf size reduction on the youngest leaves of the main stem and/or at least one branch. At Vea, genotypes carrying various resistance genes such as Pimplifolium (*ty-5* and *Ty-6*) appeared healthy, no observable symptoms; AVTO1429 (*Ty-2*, *Ty-3*), and AVTO1219 (*Ty-1/3*, *Ty-2*), AVTO1311 (*Ty-1/3*) expressed moderate yellowing and/or leaf curling on the youngest (top) leaves. *Pimpinellifolium* x Wosowoso (*Ty-6*) expressed mild yellowing, mosaic and/or slight leaf curling on youngest leaves, severe symptoms at 60 DAT.

Table 5. 6: Severity ratings of TYLCD on infected tomato at 30 DATS, 45 DAT and 60 DAT at Akumadan and Vea

	Akumadan				**Vea**		
Genotypes	**30 DAT**	**45 DAT**	**60 DAT**	**Genotypes**	**30 DAT**	**45 DAT**	**60 DAT**
P005A	2	4	5	Power Rano	3	4	4
Power Rano	2	4	5	AVTO1311	1	2	4
Peto Mech	2	3	5	AVTO1424	1	2	4
AVTO1219	3	4	5	AVTO9802	1	2	4
AVTO1311	2	4	5	AVTO0301	3	4	4
AVTO1429	2	4	5	AVTO1350	2	3	4
AVTO1424	2	4	5	LA4440a	2	2	4
AVTO9802	2	4	5	Tomato T0 687 F_1 Hybrid	1	3	4
AVTO0301	4	4	5	Tapiche	2	3	4
AVTO1350	2	4	5	Lorry Tyre	2	3	4
Tomato T0 687 F_1 Hybrid	2	5	5	Power	2	3	4
Tapiche	3	4	5	P005A	2	2	3
Lorry Tyre	3	5	5	P005B	2	3	3
Nadira	2	4	5	Peto Mech	2	3	3
Power	3	3	5	AVTO1219	1	1	3
Thorgal	3	4	5	AVTO1429	1	1	3
Wosowoso	2	4	5	*Pimpinellifolium* x Wosowoso	1	2	3
P005B	2	4	4	Nadira	2	2	3
LA4440a	2	4	4	Thorgal	2	3	3
Pimpinellifolium x Wosowoso	2	2	3	Wosowoso	2	2	3
Pimplifolium	1	2	2	Pimplifolium	1	1	1

1=Healthy, 2=Very mild, 3=Mild, 4=Moderate, 5=Severe, 6=Very severe

5.3.2.4 Yield and Yield Component traits at Akumadan Dam site

There were significant variations among accessions for the yield and yield component traits studied. Number of fruits per plant, average fruit weight, fruit length and fruit diameter were significant at 5% at both locations. In Akumadan, the highest fruits per plant was produced by Pimplifolium (58 fruits/per plant), followed by *Pimpinellifolium* x Wosowoso (48 fruits/plant), Power Rano (6 fruits/plant) and P005A with 6 fruits/plant (Table 5.7). Although Pimplifolium recorded the highest number of fruits, it had the lowest weight per fruit (7.65 g) with Thorgal being the heaviest fruit (72.64 g). Genotypes *Pimpinellifolium* x Wosowoso, Pimplifolium and P005A gave the maximum yields of 26.58 t/ha, 24.06 t/ha and 17.96 t/ha respectively. Lorry Tyre was the widest fruit (29.02 mm) and AVTO1219 was the least in diameter (12.65 mm). Nadira had the highest fruit length of 27.69mm and Pimplifolium had the lowest fruit length of 10.12 mm.

Table 5. 7: Means of yield and yield component traits of 21 tomato genotypes evaluated at Akumadan

Genotype	**No. of fruits/ Plant**	**Fruit weight (g)**	**Yield (t/ha)**	**Fruit Diameter (mm)**	**Fruit Length (mm)**
AVTO1219	1	8.85	0.06	12.65	14.79
AVTO1311	1	29.56	0.5	19.42	19.95
AVTO1429	1	52.63	2.22	23.58	22.77
AVTO1424	1	31.99	0.42	19.32	20.63
AVTO0301	1	19.24	0.41	18.02	21.36
AVTO1350	1	13.35	0.4	14.8	19.37
Tomato T0 687 F_1 Hybrid	1	56.6	3.95	21.81	26.62
Tapiche	1	70.69	4.28	23.18	27.17
AVTO9802	2	19.7	1.87	17.39	19.34
Lorry Tyre	2	68.66	6.45	29.02	25.06
Power	2	50.04	5.7	25.63	20.65
Peto Mech	3	31.73	4.87	18.26	22.73
LA4440a	3	19.08	3.19	21.93	17.09
Thorgal	3	72.64	11.71	27.39	21.36
P005B	4	54.32	10.74	22.8	24.62
Nadira	4	44.04	6.81	21	27.69
Wosowoso	4	56.26	10.93	27.67	18.76
P005A	6	68.36	17.97	27.61	20.37
Power Rano	6	44.62	13.24	24	19.15
Pimpinellifolium x Wosowoso	48	10.29	26.58	14.3	10.83
Pimplifolium	58	7.65	24.06	12.98	10.12
Mean	7	39.5	7.45	21.08	20.5
L.s.d (5%)	16	22.84	12.286	4.98	3.716

5.3.2.5 Yield and Yield Components traits at Vea Irrigation site

At Vea, Pimplifolium produced the highest number of fruits per plant (37 fruits/plant), with LA4440a producing the lowest (2 fruit/plant). Pimplifolium recorded the lowest average fruit weight (7.15 g) while Lorry Tyre recorded the highest average fruit weight (103.29 g). Power gave the highest fruit yield (48.74 t/ha) followed by B (34.14 t/ha) and Nadira (30.79 t/ha). The widest diameter was recorded in Lorry Tyre (59.75mm) and the Nadira had the longest fruit length of 51.47 mm (Table 5.8).

Table 5. 8: Means of yield and yield component traits of 21 tomato genotypes evaluated at Vea

Genotype	No. of fruits/ plant	Fruit weight (g)	Yield (t/ha)	Fruit diameter (mm)	Fruit Length (mm)
LA4440a	2	17.42	1.96	12.24	7.94
AVTO1219	4	34.64	6.93	42.07	41.88
AVTO1311	4	31.88	6.96	42.4	41.75
AVTO0301	4	31.51	6.72	36.37	45.06
P005A	5	55.21	14.07	26.88	20.82
AVTO1424	5	40.99	9.89	39.02	38.55
Lorry Tyre	5	103.29	26.07	59.75	39.81
AVTO1429	6	61.41	22.47	45.7	39.71
AVTO9802	6	44.35	13.16	39.46	48.63
AVTO1350	6	37.7	11.9	34.84	42.35
Thorgal	6	52.16	16.4	45.58	35.75
Wosowoso	6	89.96	25.72	56.31	35.48
Peto Mech	7	50.4	17.91	41.69	48.46
Tapiche	7	55.25	17.95	44.24	46.77
P005B	10	70.13	34.14	44.46	44.91
Power Rano	10	48.86	24.24	44.36	36.61
Tomato T0 687 F_1 Hybrid	11	41.79	22.66	41.71	50.39
Nadira	11	59.31	30.79	43.79	51.47
Power	13	78.14	48.74	56.09	42.98
Pimpinellifolium x Wosowoso	17	10.72	8.45	37.6	34.88
Pimplifolium	37	7.15	12.9	23.07	30.17
Mean	9	48.7	18.1	40.8	39.3
L.s.d (5%)	5	20.65	12.826	13.68	16.31

5.3.3 PCR amplification of TYLCV DNA in infected tomato samples

5.3.3.1 TYLCV DNA in infected Samples in Akumadan Dam site

The primer pair AV494/AC1048 F/R detected TYLCV DNA in infected samples; C-1 (Power Rano), 153 (LA4440a), G9 (Lorry Tyre), A (P005A), R (Power) collected from the disease trial in Akumadan and the amplicon size was ~550 bp (Plate 5.6). The PTYv787/PTYc1121 F/R primer pair also amplified 300 bp in C-1 (Power Rano), N (Nadira), G153 (LA440a), TH (Thorgal), R (Power), G5 (Pimplifolium) and D8 (AVTO030). Genotypes G153 (LA4440a), TH (Thorgal), R (Power) and G5 (Pimplifolium) exhibited multiple bands (Plate 5.7). The amplicon (~600 bp) for GHF/GHR primer was amplified in genotypes C-1 (Power Rano), D1 (AVTO1219), G153

(LA4440a), TH (Thorgal), A (P005A), G9 (Lorry Tyre), R (Power), G5 (Pimplifolium) and D8 (AVTO030) (Plate 5.8). Although genotypes D2 (AVTO1311), D4 (AVTO1429), D6 (AVTO9802), D5 (AVTO1424), C-2 (Peto Mech), B (P005A) and G48 (Tapiche) expressed TYLCV symptoms in the field, none of the primer sets amplified viral DNA in them. PARc1496/PAL1v1978 and KF/KR did not amplify TYLCV DNA in any of the infected samples collected.

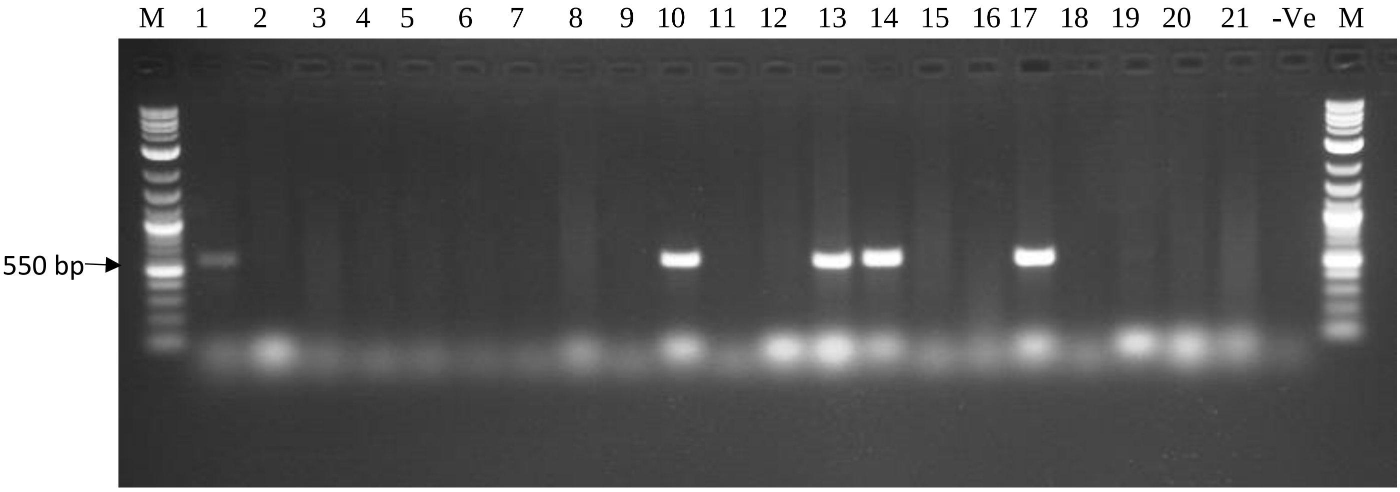

Plate 5. 6: PCR amplification products of TYLCV DNA from 21 tomato accessions using AV494/AC1048F/R primer pair. Lanes: 1=C-1, 2=D1, 3=D2, 4=C-2, 5=D-4, 6=D6, 7=D5, N=8, 9=G48, 10=G153, 11=121, 12=TH, 13=A, 14=G9, 15=W, 16=B, 17= R, 18=S1, 19= G5, 20=D9, 21=D8; M=2- Log DNA ladder; -Ve = Negative control (sterile nuclease free water)

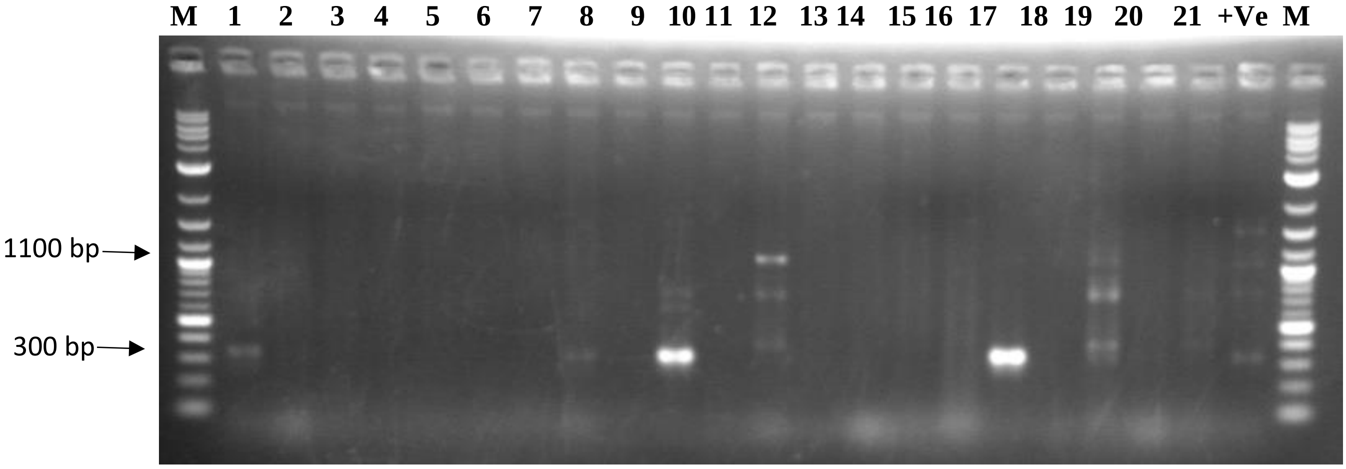

Plate 5. 7: PCR amplification products of TYLCV DNA from 21 tomato accessions using PTYv787/PTYc1121 F/R primer pair. Lanes: 1=C-1, 2=D1, 3=D2, 4=C-2, 5=D-4, 6=D6, 7=D5, N=8, 9=S2, 10=G153, 11=121, 12=TH, 13=A, 14=G9, 15=W, 16=B, 17= R, 18=S1,19= G5, 20=D9, 21=D8; M=2- Log DNA ladder; +Ve = Positive control obtained from infected sample from farmers' field in Akumadan

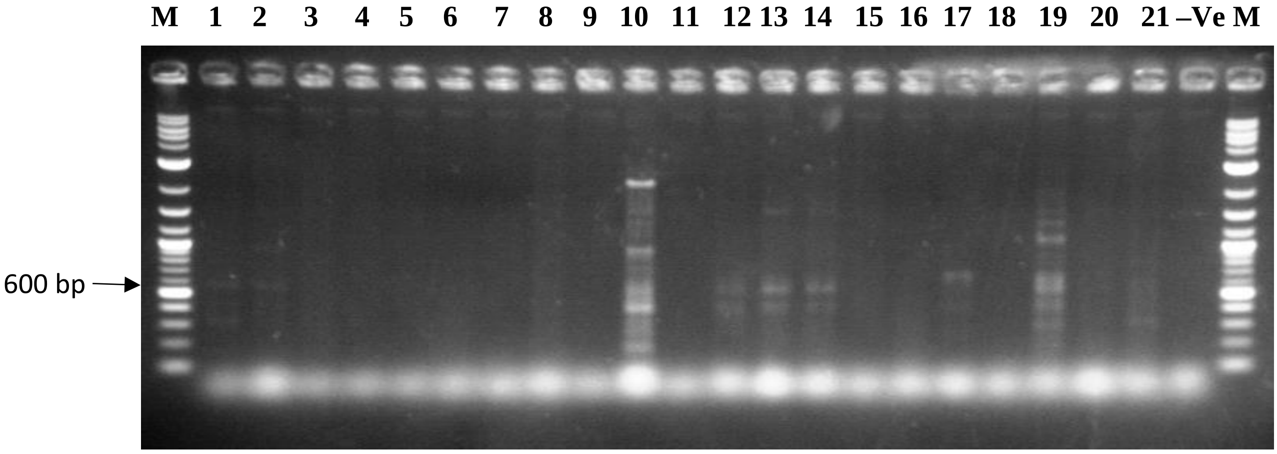

Plate 5. 8: PCR amplification products of TYLCV DNA from 21 tomato accessions using GHF/GHR primer pair. Lanes: 1=C-1, 2=D1, 3=D2, 4=C-2, 5=D-4, 6=D6, 7=D5, N=8, 9=S2, 10=G153, 11=121, 12=TH, 13=A, 14=G9, 15=W, 16=B, 17= R, 18=S1, 19=G5, 20=D9, 21=D8; M=2- Log DNA ladder; -Ve = Negative control (sterile nuclease free water).

5.3.3.2 TYLCV DNA in infected Samples in Vea Irrigation site

Two primers amplified the viral DNA in the infected samples collected from the Vea irrigation site. Primer AV494/AC1048 F/R gave an amplicon size of about ~75 bp in samples D4 (AVTO1429), N (Nadira), G153 (LA4440a), A, G9 (Lorry Tyre) in addition to ~550 bp in genotypes A (P005A) and G9 (Lorry Tyre) (Plate 5.9 and Plate 5.10). PTYv787/PTYc1121 F/R amplified double bands in two samples; D4 (AVTO1429) and D6 (AVTO9802) (Plate 5.11) out of the 21 infected samples. Primers PARc1496/PAL1v1978, GHF/R and KF/KR did not produce any amplicon when used to screen infected samples collected from Vea.

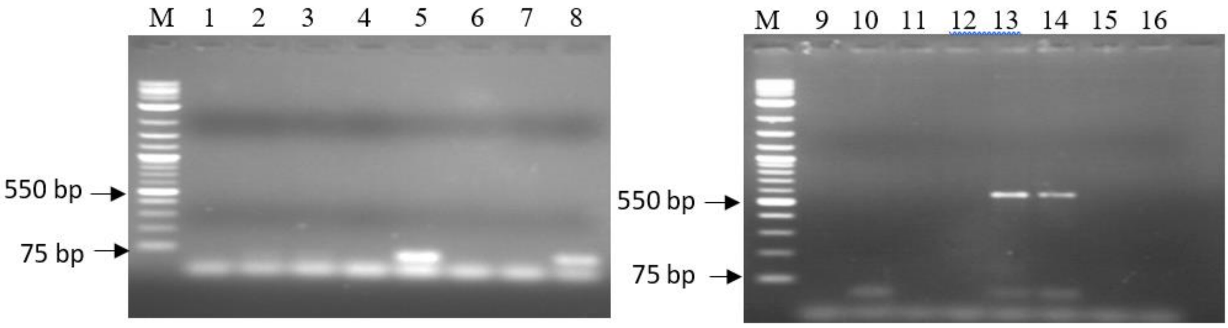

Plate 5. 9: PCR amplification products of TYLCV DNA from 21 tomato accessions using AV494/AC1048 F/R primer pair. Lanes: 1=C-1, 2=D1, 3=D2, 4=C-2, 5=D-4, 6=D6, 7=D5, N=8, 9=S2, 10=G153, 11=121, 12=TH, 13=A, 14=G9, 15=W, 16=B

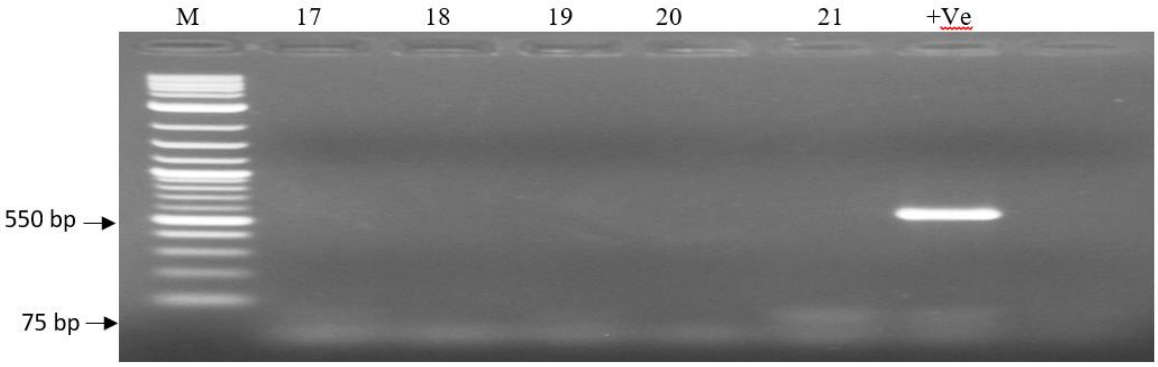

Plate 5. 10: PCR amplification products of TYLCV DNA from 21 tomato germplasm using AV494/AC1048F/R primer pair. Lanes: 17= R, 18=S1, 19= G5, 20=D9, 21=D8; M=2-Log DNA ladder; +Ve = Positive control obtained from infected samples from farmers' field in Akumadan

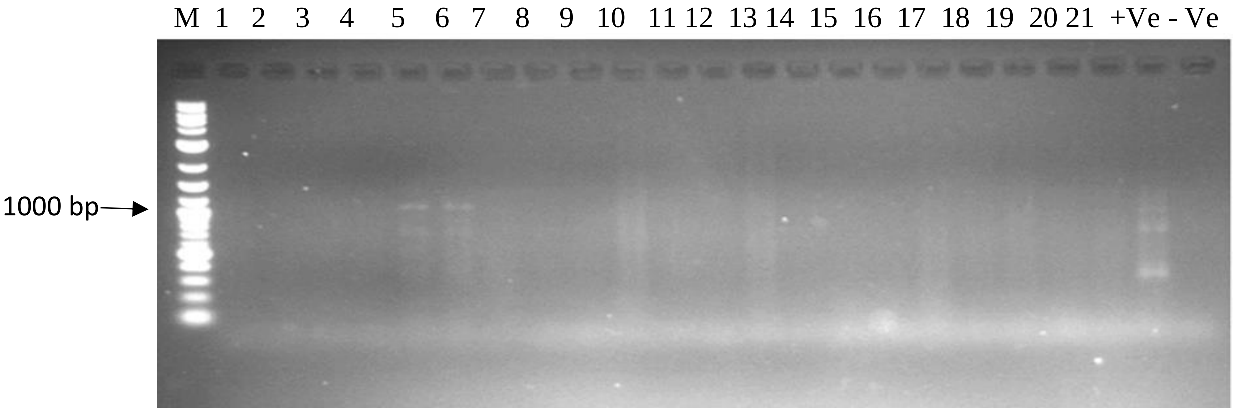

Plate 5. 11: PCR amplification products of TYLCV DNA from 21 tomato germplasm using PTYv787/PTYc1121 F/R primer pair. Lanes: 1=C-1, 2=D1, 3=D2, 4=C-2, 5=D-4, 6=D6, 7=D5, N=8, 9=S2, 10=G153, 11=121, 12=Th, 13=A, 14=G9, 15=W, 16=B, 17= R, 18=S1, G5, 20=D9, 21=D8; M=2- Log DNA ladder; +Ve = Positive control obtained from infected sample from farmers' field in Akumadan; -Ve = Negative control (sterile nuclease free water)

The viral DNA marker analyses of the infected samples collected from both Akumadan and Vea are summarized in Table 5.9.

Table 5. 9: Scores of 5 TYLCV detection primers in 21 tomato genotypes

Genotype	**Primers**						
	AV4/AC		**PTY/PTYc**		**GHF/GHR**	**PAR/PAL**	**KF/KR**
	Aku	**Vea**	**Aku**	**Vea**	**Akum**	**Aku/Vea**	**Aku/Vea**
P005A	+	+	–	–	+	–	–
P005B	–	–	–	–	–	–	–
Power Rano	+	–	+	–	+	–	–
Peto Mech	–	–	–	–	–	–	–
AVTO1219	–	–	–	–	+	–	–
AVTO1311	–	–	–	–	–	–	–
AVTO1429	–	+	–	+	–	–	–
AVTO1424	–	–	–	–	–	–	–
AVTO1350	–	–	–	+	–	–	–
AVTO030	–	+	+	–	+	–	–
AVTO1350	–	–	–	–	–	–	–
Pimpinellifolium x Wosowoso	–	–	–	–	–	–	–
LA4440a	+	+	+	–	+	–	–
Pimplifolium	–	–	+	–	+	–	–
Lorry Tyre	+	+	–	–	+	–	–
Nadira	–	+	+	–	–	–	–
Power	+	–	-	–	+	–	–
Tomato T0 687 F_1 Hybrid	–	–	–	–	–	–	–
Tapiche	–	–		–	–	–	–
Thorgal	–	–	+	–	+	–	–
Wosowoso	–	–	–	–	–	–	–

Aku=Akumadan; AV4/AC=AV494/AC1048 F/R; PTY/PTYc=PTYv787/PTYc1121 F/R; PAR/PAL=PARc1496/PAL1v1978

5.4 Discussion

5.4 .1 Confirmation and identification of TYLCD resistance genes

The deployment of tomato cultivars with resistance has been considered to be the most effective strategy to reduce yield losses caused by viral diseases (Lapidot and Friedman, 2002). The PCR carried out in the study confirmed the presence of the TYLCD genes in accessions AVTO1219 (*Ty*-*3*), AVTO1311 (*Ty-2*, *Ty*-*3*), AVTO1429 (*Ty-2*, *Ty*-*3*) and identified *ty-5*, *Ty-6* in Pimplifolium and Ty-6 in *Pimpinellifolium* x Wosowoso. The expected band sizes for *ty-5* are 170/175 bp but in the current study, the band sizes amplified were approximately 200/230 bp. The amplified band size of approximately 230 bp amplified in this study could be due to a variant of the *ty*-*5* gene present in Pimplifolium. Chen et al (2015) reported that in *ty*-*5* susceptible *S. chilense*, AVRDC–TM273 yielded different banding patterns than in S. *lycopersicum* and attributed it to the presence of different alleles at this locus in *S. chilense* than in cultivated tomato.

Over the years the main objective of several breeding programmes has been the effectiveness of different resistance sources in different locations and their response to different begomoviruses (Picó *et al.*, 1999). All the 21 genotypes screened showed variation for TYLCD incidence and severity at both locations. The symptom expression varied among the genotypes, growth stage and location. Incidence and severity were lower at early stages of growth but severe at later stages of growth. Accessions expressed severe symptoms in Akumadan than at Vea due to the high disease pressure in Akumadan. TYLCD resistance in cultivars can breakdown occasionally under high disease pressure (Zamir *et al.*, 1994) and this might be the reason why although some accessions have resistance genes but expressed severe disease symptoms in Akumadan. The same accession had different severity score in Akumadan and Vea. Lapidot *et al*. (2000) reported that the variability in screening conditions and assays leads to contradictory results where different resistance levels have been attributed to the same genetic sources. Pimplifolium had 2 and 1 severity score and Vea respectively. This is an indication of a high level of resistance to the

TYLCD at both locations. Hanson *et al*. (2016) reported that lines from CLN3241 which expressed disease severity score of less than 3 had a high level of resistance to TYLCTHV and ToLCTWV. This is consistent with the findings of Asare-Bediako *et al*. (2017) who reported that the severity of TYLCD in the Central region was lower because farmers were growing *Pimpinellifolium* or improved form of *Pimpinellifolium*.

Due to the high level of resistance of Pimplifolium, it produced the highest yield compared to all the others in Akumadan. Although Pimplifolium showed TYLCD symptoms in Akumadan and two sets of primer pairs amplified the viral DNA in the samples collected, it was effective against the strains of the virus in both locations. The presence of the TYLCD resistance genes in AVTO1219, AVTO1311 and AVTO1429 did not reflect in their reaction to the local strain of TYLCV in Akumadan. However, at Vea irrigation site AVTO1219, AVTO1311 and AVTO1429 were more tolerant of the TYLCD than the most preferred variety (Peto Mech). TYLCD resistance genes present a range of resistance levels, differential responses to isolates, strains and species (Prasanna *et al*., 2015).

AV494/AC1048 F/R amplified a band size of ~550 bp in some infected samples from both locations. The results from this study, however, is at variance with the band size of 2500 bp reported by Osei *et al*. (2012). In addition to the ~550 bp, AV494/AC1048 F/R also amplified 75 bp in some of the samples collected from Vea but not Akumadan. This band size was not reported in previous studies. The band size of 300 bp produced by PTYv787/PTYc1121 F/R primer pair is in agreement with the works of Nakhla *et al*., (1993) and Asare-Bediako *et al*. (2017). Primers PARc1496/PAL1v1978 and KF/KR did not produce any amplicon in the samples collected from Akumadan. In addition to these two primers, GHF/GHR did not also detect viral DNA in the samples collected from Vea and this could be attributed to the absence of the particular viral strains, low viral concentration or absence of complementary sequence at primer annealing sites (Potter, 2003; Rotbi *et al*., 2015).

5.5 Conclusion

This is the first report on the detection of *ty*-*5* and *Ty*-*6* in Pimplifolium and also *Ty*-*6* in *Pimpinellifolium* x Wosowoso in Ghana. Even though two of the viral primers detected viral DNA in Pimplifolium, it expressed a high level of resistance at both locations and therefore *ty*-*5* and *Ty*-*6* can be introgressed into the locally adapted cultivars. Although the accessions (AVTO1219, AVTO1311, AVTO1429) with different TYLCD resistance genes obtained from AVRDC expressed severe symptoms in Akumadan and mild symptoms at Vea, the genes can be pyramided together with the *ty*-*5* and Ty 6 genes amplified in Pimplifolium into locally adapted cultivars.

CHAPTER SIX

6.0 Combining Ability Analysis for Fruit Quality and Yield in tomato (*Solanum lycopersicum* L.)

6.1 Introduction

Tomato production in Ghana is dominated by unadapted imported hybrids or farmer saved seeds (Osei *at al*., 2015). Farmers are unable to realize the full potential of these imported hybrids due to the unadaptability to the local growing conditions. Additionally, fruits produced from farmers' cultivars are generally watery, poor in colour and have poor shelf life (Robinson and Kollavali, 2010). Tomato production in Ghana is plagued with high incidence of pests and diseases; particularly Tomato Yellow Leaf Curl Virus Disease (TYLCD). TYLCD have been reported to cause severe yield loss in most important tomato production areas throughout the country (Horna *et al*., 2006; Osei *et al*., 2012; Duodo, 2014).

To mitigate against these tomato production constraints, a breeding strategy that will combine increased yield, fruit quality together with TYLCD resistance will be very useful. This will involve a hybridization programme that will utilize lines with different Tomato Yellow Leaf Curl Disease Resistance genes (TYLCD resistance genes), some level of fruit quality traits with locally adapted cultivars. It is important to combine all these traits in a single variety because high yielding and quality fruit will not be realized when TYLCD is prevalent.

In estimating the potential of pure lines for hybrid development, the general and specific combining abilities (GCA and SCA) are the most important indicators (Zengin *et al*., 2015). North Carolina II analysis provides information on GCA and SCA effects of parents and can be used to estimate the various types of gene action.

The objective of this work was to identify lines with good general combining abilities and specific crosses that combine well for fruit quality, yield and TYLCD resistance.

6.2 Materials and Methods

6.2.1 Plant Materials

The genetic materials used for this study were made up of six locally-adapted genotypes and three exotic lines. The locally-adapted genotypes include Lorry Tyre, Peto Mech, Power Rano, Power, Wosowoso and Pimplifolium sourced from the Plant Genetic Resource Research Institute (PGRRI), University of Ghana, farmers and Agrimat Limited. Pimplifolium and its improved forms have been reported to have high tolerance to TYLCD in Ghana. The other locally-adapted genotypes are susceptible to the TYLCD. The exotic lines have different combination of TYLCD resistance genes (AVTO1219 – *Ty-1*/T-y3, *Ty-2*; AVTO1311 – *Ty-1/3* and AVTO1429- *Ty-2*, *Ty-3*) as well as good fruit characteristics. They were sourced from the World Vegetable Centre in Taiwan (AVRDC).

6.2.2 Greenhouse Experiment: Crossing Block

The crossing block was made up of five females and four males. The five females included Lorry Tyre, Peto Mech, Power Rano, Power and Wosowoso. The males included three AVTO1219, AVTO1311 AVTO142) and the local Pimplifolium. The mating design used was North Carolina II design (5 x 4 NCII design).

6.2.3 Confirmation of TYLCD resistance genes in F_1 Plants

Leaf samples of 8 plants were harvested from each set of the 20 F_1 plants and stored on ice and transported to the Virology Department of the Noguchi Memorial Institute for Medical Research, University of Ghana. DNA was extracted from the plant samples using QIAamp DNA Mini kit (Qiagen, Hilden, Germany). The quantity and quality of the DNA were checked using the Thermo Scientific NanoDrop (2000C spectrophotometer). The presence of the TYLCD resistance genes (TYLCD resistance genes) in the 20 F_1 hybrids were confirmed by Polymerase Chain reaction (PCR) using the markers listed below (Table 6.1).

The PCR conditions are presented in Table 6.2. The PCR products were separated on ethidium bromide stained 1.5% agarose gel and visualized under UV light.

Table 6. 1: Primers used for the Amplification of TYLCD resistance genes in tomato leaf samples

Ty gene	**Marker name**	**Sequence**	**PCR product (bp)**
Ty-2	P1-16	F: CACACATATCCTCTATCCTATTAGCTG R: CGGAGCTGAATTGTATAAACACG	Resistant: 300 Susceptible: 600
Ty-3	P6-25	F: GGT AGT GGA AAT GAT GCTGCTC R: GCT CTG CCT ATT GTC CCA TAT ATA ACC	Resistant: 450, 650 Susceptible: 320
Ty5	TM273	F: GGTGCTCATGGATAGCTTAC R: CTATATAGGCGATAGCACCAC	Resistance:175 Susceptible: 170

Table 6. 2: PCR conditions for primers used the amplification of TYLCD resistance genes.

P1-16 (Hanson, 2016)		P6-25 (Ji *et al*., 2010)		TM273 (Hanson, 2016)	
Temp. (°C)	**Time(min)**	**Temp.(°C)**	**Time(min)**	**Temp.(°C)**	**Time(min)**
1. 95	10:00	1. 94	4:00	1. 95	5:00
2. 94	0:30	2. 94 ^{0}C	00:30 (35x)	2. 94	0:30 (34 x)
(34x)		3. 53 ^{0}C	1:00	3. 55	0:30
3. 55	0:45	4. 72 ^{0}C	1:00	4. 72	0:30
4. 72	0:45	5. 72 ^{0}C	10:00	5. 72	5:00
5. 72	5:00			6. 20	∞
6. 20	∞				

6.2.4 Field Experiment: Experimental Design and Field Layout

The F_1s (20) together with their parents (9) were laid out in a Randomized Complete Block Design (RCBD) with three (3) replication. Each genotype was planted in two rows with each row consisting of 10 plants. The inter-row spacing was 1m and intra-row spacing was a row 0.5 m.

6.2.4.1 Nursery and Agronomic practices

Three seeds were sown in each cell of the nursery trays and thinned to one per cell after 14 days of nursing. Thirty-day-old seedlings were transplanted to the field. At two weeks after transplanting, N.P.K. 15-151-15 was applied at the rate of 8-10g per plant. At four weeks after transplanting, Sulphate of Ammonia was applied at the rate of 5g per plant. Sulphate of Ammonia was applied at the rate of 5g per plant. Calcium nitrate (Fertigation grade) was applied at fruiting at the rate of 150 g/L.

6.2.4.2 Experimental site

The crosses were carried OUT in a greenhouse at the West Africa Centre for Crop Improvement (WACCI) at the University of Ghana Farms, Legon from June - October 2017. The crosses together with their parents were evaluated from February-May 2018 in the field at the University of Ghana Farms. The farm is between latitude 5^o 38 45 N and longitude 00^o 11 13 E. The soil type was Haatso Series with an average rainfall of 809 mm. The minimum temperature was 23.8 ^oC and the maximum temperature of 31.2^oC.

6.2.4.3 Data collection

Data on vegetative, reproductive, yield and fruit quality were collected based on the tomato IPGRI and the UPOV tomato descriptors. The following vegetative and reproductive data were collected: foliage density, leaf coverage, days to first flowering, number of primary branches, plant height at maturity (cm) and days to first fruit set. The following yield and yield component data were also measured: fruit length (mm), fruit diameter (mm), number of fruits per plant, fruit weight (g) per plant and yield per plant (g). The fruit quality data collected also include fruit green shoulders, fruit green stripes, fruit hardness (N/cm^2), pericarp thickness (mm), number of locules, ribbing, pH and shelf life.

6.2.4.4 Data Analysis

Analysis of Variance was estimated using GenStat version 12th Edition. The General and Specific Combining Ability were estimated using Analysis of Genetic Designs in R (AGD-R) version 3.0 (2015-08-28) developed by CIMMYT.

Mid-parent heterosis and heterosis over better-parent (Heterobeltiosis) were calculated in terms of increase or decrease of hybrid over average of both parents or better-parent (Hayes *et al*., 1995).

Mid parent heterosis (%) = $[(F_1\text{-}MP)/MP] \times 100$

Heterobeltiosis (%) = $[(F_1\text{-}BP)/BP] \times 100$

6.3 Results

6.3.1 Confirmation of Tomato Yellow Leaf Curl Virus Resistance genes (TYLCD resistance genes) in 20 F_1 hybrids

Primer P1-16 amplified resistance band (300 bp) or heterozygous bands (300 and 600 bp) in 57% of 5 different F_1 hybrids with D4 (AVTO1429) as the donor parent (Plate 6.1). Primer P6-25 also amplified 81% heterozygous bands (320 bp and 420 bp) in the 5 F_1 hybrids with D4 (AVTO1429) as the donor parent (Plate 6.2). Primer TM273 confirmed the heterozygous state of *ty-5* genes in 67% of 5 different hybrids with the Pimplifolium as donor parent (Plate 6.3).

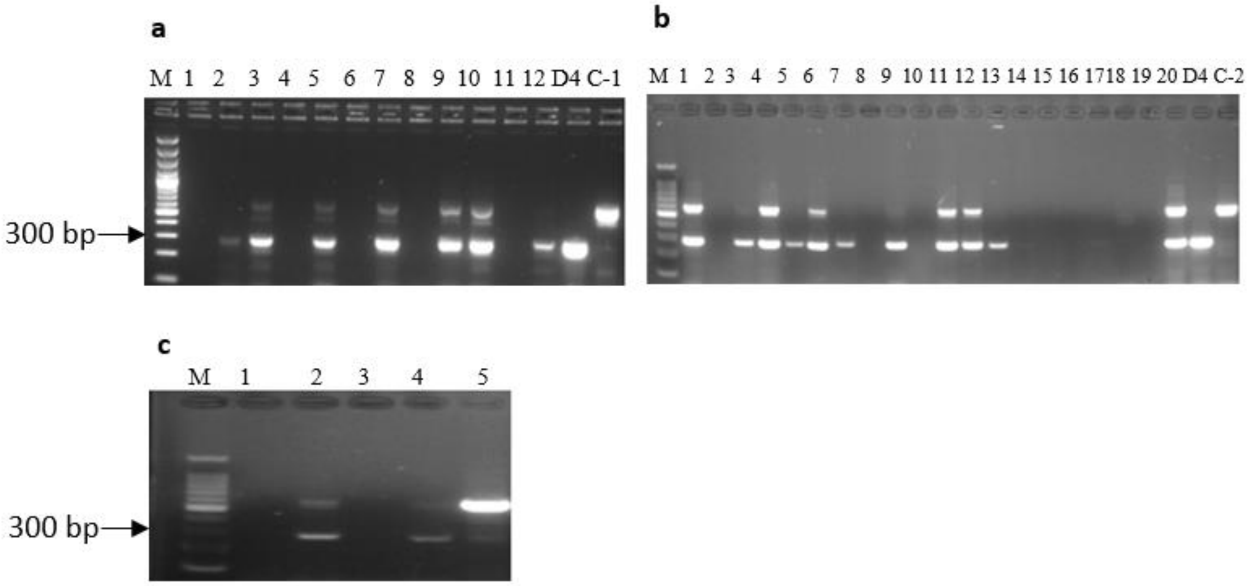

Plate 6. 1: PCR amplification products of *Ty-2* gene obtained from F_1 hybrids using P1-16 F/R primer pair. Lanes: (a) 1-8 = C-1 x D4, 9-12 = C-2 x D4; (b) 1-4 = C-2 x D4, 5-9 = G9 x D4, 10-17 = W x D4, 18-20 = R x D4; (c) 1-5 = D4 = AVTO1429 (Resistant Male Parent) C-1= Power Rano and C-2 = Peto Mech (Susceptible Female Parents); M=100 kb DNA ladder. Susceptible Band: 600 bp, Resistant 300 bp

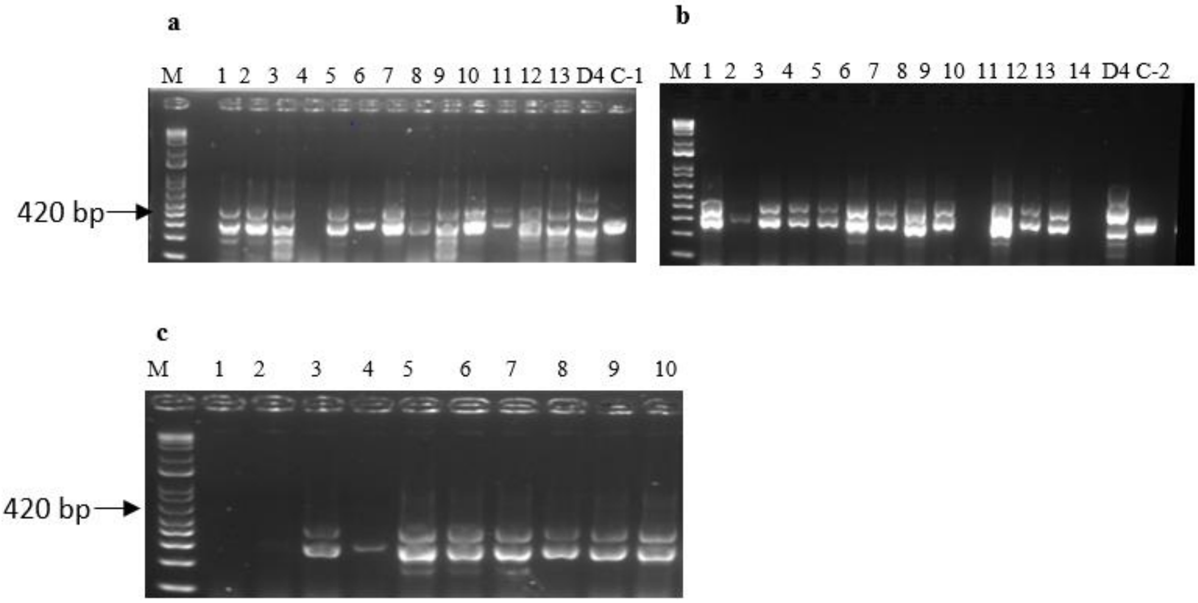

Plate 6. 2: PCR amplification products of *Ty-3* gene obtained from F$_1$ hybrids using P6-25 F/R primer pair. Lanes: (a) 1-8 = C-1 x D4, 9-13 = C-2 x D4; (b) 1-3 = C-2 x D4, 4-8 = G9 x D4, 9-14 = W x D4; (c) 1-2 = W x D4, 3-9 = R x D4, D4 = AVTO1429 (Resistant Male Parent) C-1= Power Rano and C-2 = Peto Mech (Susceptible Female Parents); M=100 kb DNA ladder. Susceptible Band: 320 bp, Resistant 420 bp

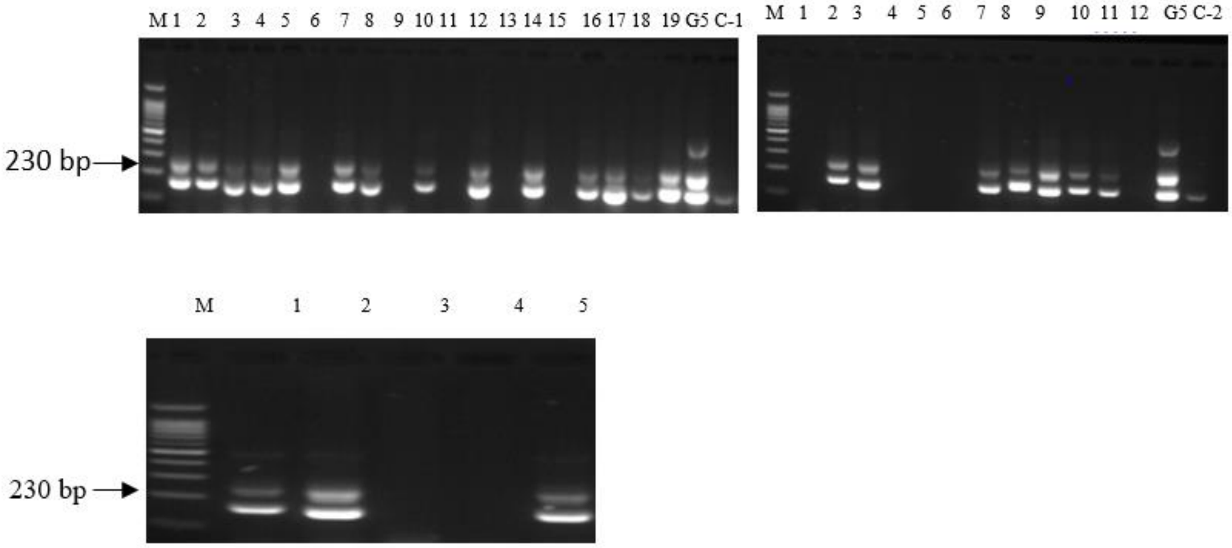

Plate 6. 3: PCR amplification products of *ty-5* gene obtained from F$_1$ hybrids using TM273 F/R primer pair. Lanes: (a) 1-8 = C-1 x G5, 9-15 = C-2 x G5, 16-19 = G9 x G5; (b) 1-4 = G9 x G5, 5-9 = W x G5, 10-12 = R x G5; (c) 1-6 = R x G5, G5 = Pimplifolium (Resistant Male Parent) C-1= Power Rano and C-2 = Peto Mech (Susceptible Female Parents); M=2- Log DNA ladder. Susceptible Band: 175 bp, Resistant 230 bp

6.3.2 Variability in parental and progeny for fruit quality and yield component traits

There were significant differences ($p < 0.01$) among the 20 hybrids and parents for vegetative, reproductive and fruit quality traits for (Table 6.3).

Table 6. 3: Mean squares for vegetative, reproductive and fruit quality traits for the 20 tomato hybrids and 9 parents evaluated

Source of variation	**df**	**Days to first flowering**	**Number of branches**	**Height at maturity (cm)**	**Days to first fruit set (mm)**	**Fruit length (mm)**	**Fruit diameter (mm)**	**Fruit/plant**	**Weight/ plant (g)**	**Yield/ plant (g)**	**Pericarp thickness (mm)**	**Fruit hardness (N/cm^2)**	**pH**	**Shelf life (days)**
Genotype	28	51.04***	2.89***	395.22***	80.76***	334.91***	284.71***	3320.16***	2095.89***	291462***	3.12***	111.15***	0.06**	47.09***
Rep	2	3.36	2.31	7.48	3.724	1.19	26.80	155.30	4.61	158417	0.42	1.465	0	11.94
Error	56	3.37	0.35	13.79	5.486		6.392	79.58	38.46	57672	0.24	2.018	0.02	5.4

***highly significant difference observed among means ($P < 0.001$); **highly significant difference observed among means ($P < 0.01$)

6.3.4 Combining ability analysis

Analysis of variance for mean square was significant for all traits studied (Tables 6.4 and Table 6.5). The narrow sense heritability (h^2) of the traits studied ranged from 0.56 for number of branches to 0.95 for fruit diameter. High heritability was observed for days to first flowering (0.84), days to first fruit set (0.81), fruit length (0.87), fruit diameter (0.95), fruit per plant (0.90) weight per fruit (0.85) and pH (0.81). Heritability was low for number of branches (0.56) and yield (0.59).

Table 6. 4: Mean squares and heritability for vegetative, reproductive and yield component traits studied among parents

Source of variation	**df**	**Days to first flowering**	**Number of branches**	**Height at maturity (cm)**	**Days to fruit set**	**fruit/plant**	**Fruit length (mm)**	**Fruit diameter (mm)**	**Weight/fruit (g)**	**Yield/plant (g)**
Rep	2	6.02	1.67	10.47	6.65	130.94	2.63	15.72	28.54	146034.90
Genotypes	19	54.15**	1.65**	402.39**	85.12**	4075.60**	308.29**	174.75**	1566.66**	220184.05**
Female (F)	4	9.65**	1.77**	1017.96**	18.46*	697.42**	203.14**	32.35**	624.14**	387287.68**
Male (M)	3	280.42**	2.87**	431.60**	418.99**	22766.16**	1484.08**	1008.08**	7870.89**	246641.87**
(M x F)	12	12.42**	1.31**	189.89**	23.87**	529.02**	49.39**	13.88	304.78**	157868.38**
Error	38	2.51	0.26	11.5	5.7	108.02	6.69	7.2	37.13	54856.06
h^2		**0.84**	**0.56**	**0.68**	**0.81**	**0.90**	**0.87**	**0.95**	**0.85**	**0.59**

*Significant difference observed among the means ($P < 0.05$); **highly significant difference observed among means ($P < 0.01$); h^2 = Narrow Sense heritability

Table 6. 5: Mean square for combining ability for fruit quality traits studied among parents

Source of variation	**Df**	**Pericarp thickness (mm)**	**Fruit hardness (N/cm^2)**	**pH**	**Shelf life (days)**
Rep	2	0.48	1.22	0.00	14.55
Genotypes	19	3.05**	67.62**	0.04*	40.09**
Female (F)	4	0.38	112.84**	0.07*	10.23
Male (M)	3	13.67**	143.70**	0.11	134.42**
M x F	12	1.29**	33.53**	0.02**	26.46**
Error	38	1.29**	33.53**	0.02**	26.46**
h^2		**0.76**	**0.67**	**0.81**	**0.65**

*Significant difference observed among the mean ($P < 0.05$); **highly significant difference observed among mean ($P < 0.01$); h^2 = Narrow Sense heritability

6.3.4.1 Estimation of General Combining Ability (GCA) effects

Male parent Pimplifolium exhibited significant negative GCA for days to first flowering, days to fruit set, fruit length, fruit diameter, fruit weight, pericarp thickness and fruit hardness. The three other males exhibited significant positive GCA for days to first flowering. Peto Mech had the highest significant negative GCA for plant height but positive GCA for fruit. AVTO1311 and Peto Mech had the highest significant positive GCA for fruit length. Again, AVTO1219 and AVTO1311 had the highest significant positive GCA for fruit diameter. On the other hand, AVTO1219 and AVTO1311 and AVTO1429 had the highest significant negative GCA for number of fruits per plant while Pimplifolium had the highest significant positive GCA for fruit per plant. AVTO1311 and AVTO1219 had the highest significant positive GCA for weight per fruit. Power Rano had the highest significant negative GCA for fruit weight. Lorry Tyre had the highest significant positive GCA for yield.

AVTO1219 and AVTO1429 had the highest significant positive GCA effect for pericarp thickness. Peto Mech had the highest positive significant GCA effect for fruit hardness. While Pimplifolium had the highest significant positive GCA effect for shelf life, AVTO1219 had the highest negatively GCA effect for shelf life (Table 6.6).

Table 6. 6: GCA effects for male and female parents for vegetative, reproductive, yield component and fruit quality traits in tomato

Parent	**Days to first flowering**	**Number of Branches**	**Height at maturity (cm)**	**Days to first fruit set**	**Fruit length (mm)**	**Fruit diameter (mm)**	**Fruit/ plant**	**Weight / fruit (g)**	**Yield /plant (g)**	**Pericarp thickness (mm)**	**Fruit hardness (N/cm^2)**	**pH**	**Shelf life (days)**
Female													
Lorry Tyre	0	0.05	5.15	0	-2.39	-0.34	2.31	-0.08	**177.17***	0	-0.88	-0.08	0
Peto Mech	0	-0.17	**-11.05***	0	**5.38***	0.54	-2.38	4.55	-43.29	0	**3.82***	0.05	0
Power Rano	0	0.09	-4.07	0	-0.64	-1.48	-0.92	**-5.72***	-107.34	0’	-0.48	0.01	0
Power	0	0.03	2.78	0	-0.48	0.86	1.27	0.33	-22.33	0	-1.23	0.04	0
Wosowoso	0	0	**7.19***	0	-1.87	0.41	-0.28	0.92	-4.21	0	-1.23	-0.01	0
SE		0.17	3.88	0	2.19	0.91	3.36	3.94	90.64	0	1.56	0.04	0
Male													
AVTO1219	**2.03***	-0.02	-1.9	2.32	3.88	**5.02****	**-16.04***	**10.83***	28.67	**0.57***	-0.47	0.01	**-2.65***
AVTO1311	**1.96***	-0.27	-3.21	**3.20***	**6.71***	**4.84****	**-22.10****	**14.04***	-49.97	0.14	1.7	0.02	-0.65
AVTO1429	**2.22***	-0.02	2.39	1.94	3.69	2.1	**-18.82****	7.94	-27.06	**0.57***	1.93	0.07	-0.04
Pimplifolium	**-6.21****	0.31	2.73	**-7.46****	**-14.27****	**-11.96****	**56.96****	**-32.82****	48.36	**-1.29****	**-3.17***	-0.1	**3.35***
SE	**0.10**	**0.22**	**2.93**	**1.19**	**2.31**	**1.10**	**6.09**	**4.93**	**63.60**	**0.26**	**1.49**	**0.04**	**1.12**

*Significant difference observed among the mean (P < 0.05); **highly significant difference observed among mean (P < 0.01). GCA = General combining ability

6.3.4.2 Estimation of Specific Combining Ability Effects

AVTO1429 x Peto Mech and Pimplifolium x Peto Mech exhibited significant positive SCA effect for days to first flowering (Table 6.7). Although AVTO1219 x Power Rano and AVTO1311 x Wosowoso displayed negative SCA effect for plant height, AVTO1219 x Wosowoso and AVTO1429 x Wosowoso showed significant positive SCA effect for plant height. AVTO1219 x Power Rano showed significant positive SCA effect for fruit length but AVTO1311 x Power Rano expressed negative SCA effect for fruit length. While Pimplifolium x Peto Mech showed negative SCA effect for number of fruits per plant, Pimplifolium x Power had the highest significant positive SCA effect for fruits per plant. AVTO1429 x Lorry Tyre exhibited positive SCA effect for weight per fruit, however, AVTO1219 x Lorry Tyre and AVTO1311 x Power Rano exhibited negative SCA effect for fruit weight per fruit. AVTO1311 x Lorry Tyre had the highest significant positive SCA for yield per plant. AVTO1219 x Power Rano, AVTO1311 x Power and AVTO1429 x Peto Mech had positive SCA effect for pericarp thickness. AVTO1311 x Peto Mech and AVTO1429 x Lorry Tyre also showed positive significant SCA effect for fruit hardness. However, only Pimplifolium x Peto Mech showed significant negative SCA to fruit hardness. AVTO1219 x Wosowoso and AVTO1429 x Peto Mech exhibited negative significant SCA effect for shelf life, AVTO1311 x Power Rano expressed positive SCA for shelf life.

Table 6. 7: Specific combining ability effects for vegetative and reproductive traits studied in tomato

Crosses	**Days to first flowering**	**Number of Branches**	**Height at maturity (cm)**	**Days to first fruit set**	**Fruit length (mm)**	**Fruit diameter (mm)**	**Fruit/ plant**	**Weight /fruit (g)**	**Yield/ plant (g)**	**Pericarp thickness (mm)**	**Fruit hardness (N/cm^2)**	**Shelf life**
AVTO1219 x Lorry Tyre	-0.72	-0.48	-2.42	0.53	-4.66	-1.63	11.49	**-15.74***	173.79	-0.61	-0.06	1.02
AVTO1219 x Peto Mech	-0.98	-0.3	-3.8	-0.68	2.64	0.48	-2.86	10.51	33.28	0.37	2.51	1.02
AVTO1219 x Power Rano	0.86	0.29	**-10.99***	0.54	**5.61***	0.81	-2.34	4.68	23.91	**0.81***	-0.59	-2.04
AVTO1219 x Power	1.12	-0.19	0.74	-0.02	1.52	0.31	-5.66	-1.7	-111.78	-0.3	-1.36	1.53
AVTO1219 x Wosowoso	0.07	0.62	**9.44***	-0.2	-4.54	0.21	-2.15	4.16	47.12	-0.09	-1.18	**-3.57***
AVTO1311 x Lorry Tyre	0.91	-0.27	-1.2	0.4	1.22	1.04	5.47	4.01	**252.75***	0.06	-4.14	-0.25
AVTO1311 x Peto Mech	-1.19	-0.37	-7.9	0.3	1.9	1.18	-1.54	10.44	-106.18	-0.42	**6.75****	-1.53
AVTO1311 x Power Rano	0.12	0.49	5.58	0.5	**-6.02***	-1.92	-1.29	**-16.66***	-217.65	-0.5	2.47	**3.32***
AVTO1311 x Power	1.17	-0.52	0.41	-0.34	0.95	-0.86	-4.25	1.01	-180.02	**0.75***	-1.65	-2.3
AVTO1311 x Wosowoso	-0.67	-0.24	**-8.74***	-0.61	2.94	0.73	-0.48	3.68	-38.85	0.16	-0.96	0.26
AVTO1429 x Lorry Tyre	-0.61	1.12	6.07	0.36	-0.84	0.33	-5.31	**12.29***	-165.081	0.1	**4.43***	1.58
AVTO1429 x Peto Mech	**3.85****	-0.57	-3.14	1.94	2.97	-0.33	1.63	-1.88	41.84	**0.70***	1.37	**-3.79***
AVTO1429 x Power Rano	-1.13	-0.51	-2.18	-1.4	1.54	-0.66	6.73	-9.08	95.5	-0.13	-1.64	-0.21
AVTO1429 x Power	-1.13	0.34	-4.86	0.28	-2.24	0.45	-2.6	3.52	-0.72	-0.3	-1.55	0.04
AVTO1429 x Wosowoso	-0.61	-0.44	**12.92***	-1.02	-0.87	0.28	-2.24	-3.45	-128.53	-0.2	0.18	2.34
Pimplifolium x Lorry Tyre	-1.84	0.06	1.99	-0.46	1.62	-0.23	11.5	-0.84	56.74	-0.21	-1.64	0.77
Pimplifolium x Peto Mech	**2.88***	-0.3	5.31	0.38	-1.53	-0.54	**-21.03***	-3.8	-46.69	0.11	**-4.51***	1.53
Pimplifolium x Power Rano	-0.53	0.56	4.08	0.11	-1.85	-0.37	-12.32	1.87	-94.54	-0.33	-1.01	1.28
Pimplifolium x Power	-0.53	0.61	6.1	-0.27	-0.77	1.36	**25.17****	-1.72	**252.41***	0.01	2.59	1.02
Pimplifolium x Wosowoso	-1.05	0.1	-7.43	-0.35	0.4	-0.62	2.07	-1.31	112.69	0.03	-0.01	-2.04
SE	**1.06**	**0.34**	**4.44**	**1.29**	**2.48**	**1.22**	**7.47**	**5.83**	**128.63**	**0.32**	**1.88**	**1.44**

*****Significant difference observed among the mean ($P < 0.05$); **highly significant difference observed among SCA of crosses ($P < 0.01$).

6.3.4.3 Relative contribution of Additive and Non-Additive Gene action to various traits in tomato

The GCA values for males ranged from 6.14% for yield to 91.08 % for fruit diameter. Again, the GCA values for females ranged from 3.75% for days to first flowering to 53.26% for plant height. Fruit diameter had the highest GCA (94.98%), followed by number of fruits per plant (91.80) and then fruit length (89.88). The least GCA was recorded for yield per plant (18.99%), followed by number of primary branches (49.89%) and shelf life (58.31%). SCA values ranged from ranged 5.02% for fruit diameter to 81.01% for yield per plant (Figure 6.8).

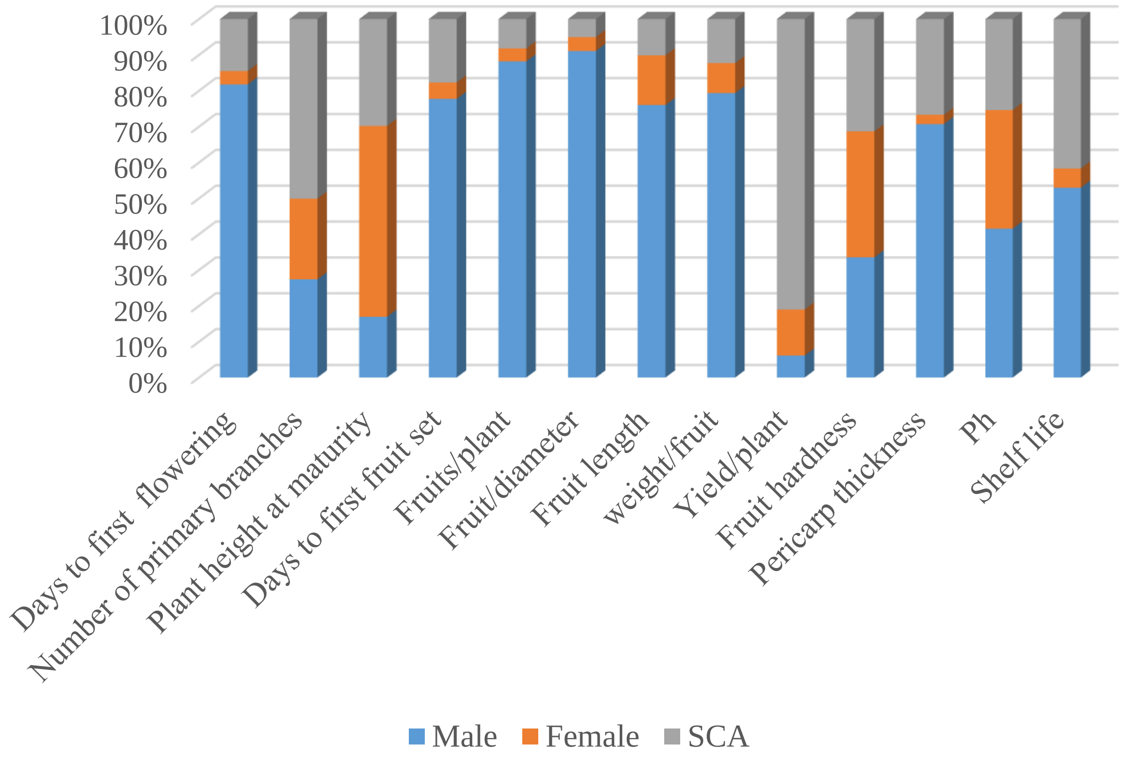

Figure 6. 1: Relative contribution of Additive and Non-Additive Genes to various traits in tomato

6.3.5 Mean performance of parents and 20 F_1 hybrids evaluated for various traits in tomato

The mean performance of parents for days to first flowering ranged from 10 days (Pimplifolium) to 23 days (AVTO1219) compared to 7 (Pimplifolium x Lorry Tyre) to 22 days (AVTO1429 x Peto Mech) in specific crosses. Mean yield of parents ranged from 301.70 g in AVTO1219 to 1283.30 g in AVTO1429, however, the mean yield of the 20 F_1 hybrids ranged from 301.10 g in Power Rano x AVTO1311 to 1306.50 g in AVTO1311 x Lorry Tyre. For fruit quality traits, the mean shelf life for parents ranged from 14 days (Wosowoso) to 28 days (Pimplifolium) while the mean shelf life of the 20 F_1 hybrids ranged from 16 days (Wosowoso x AVTO1219) to 29 days (Peto Mech x Pimplifolium) –Table 6.8.

6.3.6 Heterosis

The mid-parent heterosis ranged from -54.52% (Lorry Tyre x Pimplifolium) for days to flowering to 571.16% (Lorry Tyre x AVTO1219) for fruits per plant (Table 6.9). Heterobeltiosis ranged from -99% in fruit weight (Wososowo x AVTO142) to 383.52% for fruits per plant (Lorry Tyre x AVTO1219). Power Rano x AVTO1219 had the highest heterobeltiosis (56.03%) for pericarp thickness. For fruit hardness, Wosowoso x Pimplifolium cross exhibited the highest fruit hardness (27.21%) followed by Peto Mech x AVTO1311 (20.12%) – Table 6.10.

Table 6. 8: Mean performances of parents and the F_1s evaluated for vegetative reproductive, yield and fruit quality traits

	Number of branches	**1st flowering**	**Height at maturity**	**1st fruit set**	**Fruit length**	**Fruit diameter**	**Fruits/ plant**	**Weight**	**Yield / plant**	**Pericarp thickness**	**Fruit hardness**	**pH**	**Shelf life**
Parents													
AVTO1219	5	23	72.67	34	49.32	39.60	7.10	42.94	301.70	3.67	27.07	3.99	24
AVTO1311	6	14	47.67	24	55.45	38.16	8.30	41.28	342.60	3.55	25.20	4.20	22
AVTO1429	5	19	70.67	25	51.34	56.70	14.43	89.03	1283.80	5.20	30.90	4.43	26
Pimplifolium	6	10	75.33	20	21.42	24.47	73.77	10.28	765.20	2.16	10.40	4.00	28
Lorry Tyre	5	19	55.33	35	36.86	65.85	3.13	109.02	354.30	5.20	29.83	4.02	15
Peto Mech	4	16	55.00	24	55.29	43.76	12.17	60.25	735.40	5.44	24.73	4.15	24
Power Rano	6	12	72.00	22	32.38	51.56	23.20	54.49	1265.40	3.69	15.50	4.16	21
Power	9	14	74.00	27	35.55	54.09	16.50	75.26	1233.80	4.40	17.50	3.96	21
Wosowoso	6	11	67.00	23	36.32	60.35	7.00	88.72	623.20	3.07	9.50	4.11	14
Crosses													
Lorry Tyre x AVTO1219	6	16	73.33	29	34.53	43.68	34.33	37.04	1264.10	3.82	16.23	4.36	22
Lorry Tyre x AVTO1311	6	18	73.33	20	44.16	49.05	20.70	62.74	1306.50	4.31	14.10	4.26	23
Lorry Tyre x AVTO1429	8	17	86.67	27	38.75	44.84	10.43	66.08	689.10	4.80	23.37	4.28	26
Lorry Tyre x Pimplifolium	7	7	82.67	16	23.64	29.61	107.33	10.36	1104.50	2.51	11.87	4.13	28
Peto Mech x AVTO1219	6	16	55.67	25	50.74	48.95	11.60	71.58	828.30	5.16	23.63	4.30	22
Peto Mech x AVTO1311	5	16	50.00	28	52.71	50.23	7.20	74.70	536.00	3.66	30.27	3.89	21
Peto Mech x AVTO1429	5	22	60.67	34	50.93	44.35	14.47	54.57	785.70	5.61	24.83	4.31	19
Peto Mech x Pimplifolium	6	13	70.00	19	27.76	29.85	61.77	11.62	725.50	2.95	13.53	4.15	29
Power Rano x AVTO1219	7	18	55.00	29	48.15	47.62	13.70	54.65	749.90	5.75	16.07	4.26	18
Power Rano X AVTO1311	7	17	71.33	30	37.52	41.77	8.97	33.57	301.10	3.54	21.47	4.09	27
Power Rano x AVTO1429	6	16	68.67	22	43.25	41.65	22.33	36.09	803.90	4.48	17.37	4.05	23
Power Rano x Pimplifolium	7	8	75.67	18	21.37	28.19	74.17	7.81	588.10	2.35	12.93	4.18	29
Power x AVTO1219	6	19	74.33	27	43.59	48.91	11.73	53.45	627.00	4.24	14.50	4.28	23
Power x AVTO1311	5	19	72.67	27	45.76	46.31	7.43	59.75	443.80	5.25	16.37	4.32	20
Power x AVTO1429	7	16	72.67	28	39.04	46.29	12.80	56.50	741.50	4.25	16.70	4.20	24
Power x Pimplifolium	7	8	84.67	16	22.79	34.12	123.47	9.77	1204.80	2.81	15.97	4.25	28
Wosowoso x AVTO1219	7	17	88.00	26	35.19	48.26	14.60	60.71	888.60	4.54	14.70	4.14	16
Wosowoso x AVTO1311	6	16	67.33	26	46.67	49.17	10.63	63.37	678.20	4.45	17.10	4.27	23
Wosowoso x AVTO1429	6	17	96.00	23	39.24	45.48	11.70	49.15	563.70	4.38	18.53	4.33	27
Wosowoso x Pimplifolium	7	8	74.67	16	22.75	29.57	92.90	10.83	1008.80	2.83	13.23	3.96	24
SED	**1**	**2**	**3.03**	**2**	**2.08**	**2.06**	**7.28**	**5.06**	**196.10**	**0.40**	**1.16**	**0.12**	**2**

Table 6. 9: Mid parent and better heterosis of 20 F_1 hybrids evaluated for vegetative, reproductive, yield and yield component traits in tomato

	1st flowering		**Height at maturity**		**1st fruit set**		**Fruits/plant**		**Fruit length**		**Fruit diameter**	
Genotypes	**MPH**	**BPH**	**MPH**	**BPH**	**MPH**	**BPH**	**MPH**	**BPH**	**MPH**	**BPH**	**MPH**	**BPH**
Lorry Tyre x AVTO1219	-21.62	-14.05	14.58	32.53	-15.13	-13.87	571.16	383.52	-19.87	-29.99	17.00	-33.67
Lorry Tyre x AVTO1311	9.99	27.91	42.39	53.83	1.71	25.35	262.20	149.40	-4.32	-20.36	-5.68	-25.51
Lorry Tyre x AVTO1429	-11.49	-10.71	37.57	56.64	-11.10	5.29	18.79	-27.72	-12.13	-24.52	-26.82	-31.91
Lorry Tyre x Pimpinellifolium	-54.52	-35.43	26.54	49.41	-42.67	-21.65	179.14	45.49	-18.87	-35.87	-34.43	-55.03
Peto Mech x AVTO1219	-16.54	-29.42	-12.79	1.22	-14.93	1.40	20.39	-4.68	-2.99	-8.23	17.44	11.86
Peto Mech x AVTO1311	4.47	0.00	-2.60	4.89	16.67	15.08	-29.65	-40.84	-4.80	-4.94	22.63	14.79
Peto Mech x AVTO1429	30.05	42.50	-3.45	10.31	36.93	39.75	8.80	0.28	-4.47	-7.89	-11.71	-21.78
Peto Mech x Pimpinellifolium	-2.54	22.65	7.42	27.27	-15.77	-6.65	43.75	-16.27	-27.62	-49.79	-12.50	-31.79
Power Rano x AVTO1219	5.74	52.75	-23.96	-23.61	4.81	38.44	-9.57	-40.95	17.87	-2.37	4.48	-7.64
Power Rano X AVTO1311	31.64	44.42	19.21	49.63	32.33	1.52	-43.05	-61.34	-14.56	-32.34	-6.89	-18.99
Power Rano x AVTO1429	4.34	33.33	-3.74	-2.83	-6.38	-11.65	18.68	-3.75	3.32	-15.76	-23.06	-26.54
Power Rano x Pimpinellifolium	-25.39	-19.36	2.72	5.10	-15.19	0.00	52.98	0.54	-20.56	-34.00	-25.85	-45.33
Power x AVTO1219	1.83	33.36	1.36	2.28	-10.51	14.07	-0.59	-28.91	2.72	-11.62	4.41	-9.58
Power x AVTO1311	31.80	33.36	19.45	52.44	7.27	10.54	-40.08	-54.97	0.57	-17.48	0.40	-14.38
Power x AVTO1429	-2.05	-14.30	0.46	2.83	7.69	-18.35	-17.23	-22.42	-10.14	-23.96	-16.44	-18.36
Power x Pimpinellifolium	-31.52	-19.36	13.40	14.42	-30.02	0.00	173.56	67.37	-19.99	-35.89	-13.14	-36.92
Wosowoso x AVTO1219	1.94	52.96	26.01	31.34	-7.08	13.04	107.09	105.63	-17.82	-28.65	-3.43	-20.03
Wosowoso x AVTO1311	27.28	44.13	17.43	41.24	11.42	1.43	38.95	28.07	1.71	-15.83	-0.17	-18.53
Wosowoso x AVTO1429	11.13	47.13	39.46	43.28	-3.46	-20.00	9.19	-18.92	-10.47	-23.57	-22.29	-24.64
Wosowoso x Pimpinellifolium	-29.18	-25.75	4.93	11.45	-25.58	-30.43	130.04	25.93	-21.20	-37.36	-30.28	-51.00

MPH - Mid-parent heterosis; BPH - Heterobeltiosis

Table 6. 10: Mid parent and better heterosis of 20 F$_1$ hybrids evaluated for yield and fruit quality traits in tomato

	Weight/fruit		**Yield**		**Pericarp thickness**		**Fruit hardness**		**pH**		**Shelf life**	
Genotypes	**MPH**	**BPH**	**MPH**	**BPH**	**MPH**	**BPH**	**MPH**	**BPH**	**MPH**	**BPH**	**MPH**	**BPH**
Lorry Tyre x AVTO1219	-51.25	-66.02	285.40	256.79	-13.80	-26.44	-42.95	-45.59	0.55	0.42	12.61	-8.22
Lorry Tyre x AVTO1311	-16.51	-42.45	274.95	268.76	-1.42	-17.01	-48.76	-52.73	1.26	-1.12	20.39	1.52
Lorry Tyre x AVTO1429	-33.27	-39.39	-15.87	-46.32	-7.72	-7.75	-23.04	-24.37	-1.30	-6.03	24.22	-1.27
Lorry Tyre x Pimplifolium	-82.63	-90.50	97.32	44.34	-31.85	-51.76	-40.99	-60.21	-4.72	-8.04	29.24	0.00
Peto Mech x AVTO1219	38.73	18.80	59.73	12.63	13.24	-5.15	-8.76	-12.71	7.54	6.01	-6.96	-8.22
Peto Mech x AVTO1311	47.15	23.98	-0.56	-27.11	-18.67	-32.78	21.25	20.12	2.53	1.43	-8.70	-11.28
Peto Mech x AVTO1429	-26.89	-38.71	-22.18	-38.80	5.51	3.18	-10.73	-19.64	0.27	-3.32	-24.82	-28.19
Peto Mech x Pimplifolium	-67.05	-80.71	-3.31	-5.19	-22.29	-45.72	-22.97	-45.29	-1.75	-3.95	12.25	3.57
Power Rano x AVTO1219	12.18	0.29	-4.29	-40.74	56.33	56.03	-24.50	-40.64	9.31	7.94	-19.13	-24.66
Power Rano X AVTO1311	-29.89	-38.39	-62.55	-76.21	-2.13	-3.91	5.50	-14.80	2.48	-1.26	26.15	22.39
Power Rano x AVTO1429	-49.71	-59.46	-36.93	-37.38	0.75	-13.90	-25.13	-43.79	2.40	-3.77	-0.72	-10.27
Power Rano x Pimplifolium	-75.88	-85.67	-42.08	-53.52	-19.62	-36.26	-0.15	-16.58	-0.12	-4.88	17.02	2.39
Power x AVTO1219	-9.56	-28.98	-18.33	-49.18	5.00	-3.70	-34.93	-46.44	4.02	3.28	0.74	-5.47
Power x AVTO1311	2.54	-20.61	-43.69	-64.03	31.90	19.17	-23.33	-35.04	3.68	1.83	-8.38	-10.43
Power x AVTO1429	-31.22	-36.54	-41.09	-42.24	-11.49	-18.27	-30.99	-45.95	1.92	-2.42	0.02	-8.96
Power x Pimplifolium	-77.16	-87.02	20.54	-2.35	-14.37	-36.18	14.48	-8.74	0.63	-2.32	14.86	1.18
Wosowoso x AVTO1219	-7.78	-31.57	92.15	42.59	34.63	23.52	-19.61	-45.70	0.52	-2.52	-15.52	-32.88
Wosowoso x AVTO1311	-2.51	-28.57	40.44	8.83	34.35	25.16	-1.44	-32.14	0.99	0.40	27.28	4.48
Wosowoso x AVTO1429	-44.70	-99.65	-40.88	-56.09	6.04	-15.71	-8.27	-40.03	-0.20	-2.19	32.26	2.58
Wosowoso x Pimplifolium	-78.12	-87.79	45.32	31.83	8.40	-7.63	32.96	27.21	-7.33	-7.90	14.95	-13.11

MPH - Mid-parent heterosis; BPH - Heterobeltiosis

6.4 Discussion

6.4.1 Confirmation of TYLCD resistance gene in 20 F_1 hybrids

The Primers used for the amplification of the TYLCD resistance genes confirmed that most of the plants were hybrid since they amplified double bands in the F_1. This led to the avoidance of the selection of false hybrids that were going to be used in the F_2 population study.

A higher GCA indicates higher heritability and less environmental effects (Fasahat *et al*., 2016). Days to first flowering and first fruit is an important determinant of early yield and therefore Pimplifolium that contributed negative GCA effects for these traits is desirable for developing early maturing hybrids. This is consistent with the results obtained by Chishti *et al*. (2008) and Zengin *et al*. (2015). Height of tomato is a very important trait since tall tomato plants require staking. Staking tomato prevents tomato fruits from getting direct contact with the soil and soil microorganisms. Staking also improves aeration thereby reducing the prevalence of fungi diseases but it is labour and capital intensive. Genotypes such as Peto Mech and Wosowoso that contributed significant negative GCA for plant height at maturity can be exploited in developing short and firm stem hybrids. Zengin *et al*. (2015), however, reported positive significant GCA for height and inferred that it is a desirable trait for tomato growth. Peto Mech and AVTO1311 were good general combiners for fruit length. AVTO1219 and AVTO1311 were also good general combiners for fruit diameter. Lorry Tyre was a good combiner for yield. Pimplifolium was a good general combiner for number of fruits per plant but very poor combiner for weight per fruit. All the other males were good combiners for weight per fruit. Parents with significant positive GCA for yield components traits will be very desirable in breeding for high yielding varieties. These findings agree with (Zengin *et al*., 2015) who reported positive GCA for yield component traits.

AVTO1219 and AVTO1429 were good general combiners for pericarp thickness. Peto Mech was a good combiner for fruit hardness and Pimplifolium was a good combiner for shelf life. These genotypes will be useful in improving fruit quality.

6.4.2 Specific Combining Ability Effects

SCA effects are due to non-additive gene action which includes dominant or epistatic (Falconer 1989) and isolate best specific crosses with most desirable traits combination (Ercan and Mehmet 2005).

Cruz *et al*. (2004) indicated that to achieve a good estimate for SCA, at least one of the parents of the cross combination should show a good effect of GCA. The report of Kadams *et al*. (1999) also emphasizes that hybrid with high SCA involves one or two of both parents of good general combiners. These two reports agree with the observation that Pimplifolium was a good combiner for fruits per plant and this was evident in the specific cross whereby Pimplifolium x Power also had a positive significant SCA for fruit per plant. AVTO1311 x Lorry Tyre showed a desirable significant SCA for yield since Lorry Tyre was a good combiner for yield.

On the contrary, the study of Umar *et al*. (2017) showed that the absence of negative SCA effects for shattering score, days to 50% flowering and days to maturity indicated that the hybrids did not follow the trend where a high estimate of SCA should involve one or both parents exhibiting high GCA effect. In the present study for height at maturity, Wosowoso exhibited positive (undesirable) significant GCA for plant height at maturity, however, AVTO1311 x Wosowoso exhibited a negative significant SCA.

Traits like days to first flowering, days to first fruit set, fruits per plant are highly under additive gene control but yield was influenced by non-additive genes.

Positive heterosis for traits like yield is desired, however, negative heterosis is desired for traits like early maturity (Acquaah, 2012). A good cross for days to first flowering was Lorry Tyre x Pimplifolium. Selection of hybrids showing better heterosis over better parents for days to flowering will be useful for developing early commercial hybrids. Again, good cross Lorry Tyre x AVTO1219 for weight can be utilized in developing high yielding hybrids. This is consistent with Shende *et al.* (2012) who reported that heterobeltiosis for fruit weight breeding is the best for improving the trait in tomato.

6.5 Conclusion

The primers confirmed the introgressed *Ty-2*, *Ty-3* and *ty-5* genes. Pimplifolium can be used for improving earliness. Even though Pimplifolium had a positive significant GCA for number of fruits per plant, it had negative significant GCA for weight per fruit and therefore cannot be recommended for yield improvement. Peto Mech can be used to develop shorter plants. Lorry Tyre can be used to improve yield. Specific crosses such as AVTO1429 x Lorry Tyre had the highest significant weight and AVTO1311 x Lorry Tyre had the highest significant yield. AVTO1219, AVTO1429 and Peto Mech can be recommended for improving fruit quality. AVTO1311 x Peto Mech showed the highest positive significant SCA effect for fruit hardness. AVTO1219 x Power Rano had positive SCA for fruit thickness

This study gives an indication that both additive and non-additive gene effects were important in the inheritance of yield and fruit quality in tomato. The heterosis can be utilized in breeding for tomato fruits that combine high yielding, fruit quality and TYLCD resistance.

CHAPTER SEVEN

7.0 Segregation pattern for fruit quality and TYLCD resistance loci in tomato (*Solanum lycopersicum* L.) F_2 population

7.1 Introduction

In Ghana, tomato is the most important vegetable, however, cultivars grown have low yield, poor fruit quality and are susceptible to diseases and pests (Robinson and Kolavalli, 2010). Tomato Yellow Leaf Curl Disease (TYLCD) is the most important diseases affecting tomato and causes significant yield and quality losses in Ghana (Horna *et al*., 2006)

In order to improve on disease resistance, resistance genes from the wild tomato species have introgressed into tomato lines (Ji *et al.*, 2007; Hanson *et al*., 2016). Plant molecular genetics and genomics present useful information to breeders to make decisions on the incorporation of desirable genes into various elite cultivars (Varshney at al., 2014). Resistance genes have been mapped and molecular markers linked to traits have also been developed to carry out marker-assisted selection. Utilization of tightly linked molecular markers allows the selection of resistance gene(s) in segregating population even in the absence of disease infection (Mandoulakani *et al*., 2015). For traits controlled by single or few genes, molecular marker selection at F_2 generation reduces labour and cost that will be involved in screening a large number of genotypes in a subsequent generation (Barone *et al*. 2005). For self-pollinated crops like tomato, pedigree breeding is considered an effective method to develop pure lines. Selection can be based on best performing single plants (Acquaah, 2012).

Selection of tomato of individual plants with disease homozygous resistant alleles will be valuable in breeding for resilient commercial tomato variety. The objective of this work was to identify and select F_2 plants with homozygous TYLCD resistance gene loci.

7.2 Materials and Methods

7.2.1 Plant materials and Experimental Site

The F_2 population from Power Rano and AVTO1429 was used for this study. The parents were included in the trial. AVTO1429 had Ty-2 and Ty-3 genes while Power Rano lacks Ty-2 and Ty-3 genes.

7.2.3 Nursery and Agronomic practices

Two seeds of the F_2 population and parents were planted in each cell of a nursery tray and later thinned to one seedling per cell. Seedlings were watered twice daily and Ridomil was applied to prevent damping off disease.

7.2.4 Identification of plants with TYLCD resistance genes

Leaf samples were harvested from the 38 F_2 plants and one plant of each of the parents. Samples were sent to Ag Biotech in the USA for the amplification of the TYLCD resistance genes using SNP markers.

7.2.4 Statistical analysis

Chi-Square goodness of fit test was used to check whether the Ty-2 and Ty-3 will segregate in the 1:2:1 Mendelian ratio.

7.3 Results

For Ty-2 gene, the number of plants observed for the genotypes was 7 resistance alleles, 17 heterozygous and 14 susceptible alleles (Table 7.1).

Table 7. 1: Segregation pattern of 38 F_2 plants for obtained from Power Rano x AVTO1429 segregating for Ty-2 genes

Allele	**df**	**Observed (O)**	**Expected (E)**	**O-E**	**$(O-E)^2$**	**$(O-E)^2/E$**	**P value (0.05)**
R		7	9.5	-2.5	6.25	0.66	
HT		17	19	-2	4	0.21	
S		14	9.5	4.5	20.25	2.13	
χ^2	2					3	0.22

R=Resistance; HT=Heterozygous; S=Susceptible

For the Ty-3 gene, number of plants observed for resistance (3) and heterozygous (18) alleles was fewer than expected while the number of plants for susceptible was greater than expected (Table 7.2).

Table 7. 2: Segregation pattern of 38 F_2 plants for obtained from Power Rano x AVTO1429 segregating for ***Ty-3***

Allele	**df**	**Observed (O)**	**Expected (E)**	**O-E**	**$(O-E)^2$**	**(O-E)2/E**	**P value (0.05)**
R		3	9.5	-6.5	42.25	4.45	
HT		18	19	-1	1	0.05	
S		17	9.5	7.5	56.25	5.92	
χ^2	2					10.42	0.005

R=Resistance; HT=Heterozygous; S=Susceptible

7.4 Discussion

The *Ty-2* introgression segregation was an acceptable fit to the expected 1:2:1 ratio. *Ty-2* is a dominant resistance gene (Hanson *et al*., 2010) and therefore followed the inheritance of a single dominant gene.

The *Ty-3* introgression segregation deviated from the 1:2:1 ratio. This work collaborates with a similar work done by Gracia *et al*. (2008) who reported that among 77 F2 plants segregating for Ty-4 gene, the number of plants with resistant alleles were reduced (10) and the numbers of plants with the susceptible alleles were increased (43). This was attributed to the *Ty-4* introgression decreasing the number of viable seedlings and thus carried deleterious alleles for gamete viability, seed set or germination.

7.5 Conclusion

Seven (7) and three (3) homozygous resistant plants for *Ty-2* and *Ty-3* genes were identified. This will enable the screening of F_3 families in Tomato Yellow Leaf Curl Disease hotspot.

CHAPETR EIGHT

8.1 General Conclusion and Recommendation

TYLCD was the most important biotic stress in all the three regions. Most respondents were familiar with the symptoms of TYLCD but did not know the cause of the disease. Farmers applied different chemicals in an attempt to control TYLCD. The disease can cause more than 50% of yield loss. Farmers indicated high yielding, TYLCD resistant and have longer shelf life.

There was high level of diversity within the tomato germplasm assembled. The cluster analysis grouped the accessions into two distinct classes. The cluster I is largely made of improved varieties. The two cultivated accessions (Power Rano and Peto Mech) in Ghana were also found in cluster I. The cluster II was made largely of local cultivars. The 348 SNPs markers used were very informative in the assembled germplasm. With the exception of the lines from PGRRI and UC Davis, all the accessions had high level of heterozygosity. The UC DAVIS and BNARI accessions were good addition to the cultivars from PGGRI.

This study confirmed the presence of *Ty-2* and *Ty-3* TYLCD resistance genes in the lines from AVRDC. *ty-5* gene (larger band size than expected) and *Ty-6* gene were discovered in the Ghanaian line Pimplifolium from PGRRI and *ty-6* in G121 (Wosowoso x *Pimpinellifolium*) from BNARI. Pimplifolium expressed high level of resistance to TYLCD at both Akumadan and Vea.

Pimplifolium was a good general combiner for number of fruits per plant and shelf life. Peto Mech was a good combiner for fruit quality such as fruit length and fruit hardness. AVTO1219, AVTO1311 and AVTO1429 were good general combiners for fruit weight and AVTO1311 was also a good combiner for fruit diameter. Lorry Tyre can be used to improve yield. For specific crosses, Lorry Tyre x AVTO1311 expressed positive SCA for yield. Lorry x

AVTO1429 had the highest SCA for weight. AVTO1311 x Peto Mech had positive SCA for fruit hardness. AVTO1219 x Power Rano had positive SCA for fruit thickness.

Seven (7) and three (3) homozygous resistant plants for *Ty-2* and *Ty-3* genes were identified.

Recommendations

1. The discovered ty-5 and Ty-6 in Pimplifolium should be sequenced and compared to known ty-5 and Ty-6 genes.
2. The F_1 should be evaluated at different locations to establish their performance across environments.
3. The F_2 derived F_3 families should be evaluated in a disease hotspot to ascertain their level of resistance.

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APPENDIX

Questionnaire on Development of Tomato Yellow Leaf Curl Virus (TYLCV) Resistant Tomato (*Solanum lycopersicum* L.) varieties

Documentation of Farmers' perception on the importance and control of TYLCV disease in Upper East, Brong Ahafo and Greater Accra Regions of Ghana. Please tick [✓] where applicable.

A. BACKGROUND INFORMATION

1. Name of Region/Community ……………………………………………………..
2. Sex 1. Male [ ] 2. Female [ ]
3. Age of farmer 1. Below 18 years [ ] 2. 18 – 30 years [ ] 3. 31 – 40 [ ] 4. 41 – 50 years [ ] 5. Above 50 years
4. What is your level of education? 1. None [ ] 2. Primary [ ] 3. JHS/JSS [ ] 4. Secondary [ ] 5. Technical/Vocational [ ] 6. Tertiary [ ] 7. Others (Specify)………………………………………………………………….
5. Do you grow tomato? 1. Yes [ ] 2. No [ ]
6. If yes, how long have you been growing tomato? 1. Less than a year [ ] 2. 1-5 years [ ] 3. 6 – 10 years [ ]
7. What is the size of your land for tomato cultivation? …………………………………
8. Where do you obtain your tomato seeds? 1. Agro-seed shops [ ] 2. Saved seeds [ ] 3. Market [ ] 4. MOFA [ ] 5. Research Institutions [ ] 6. NGO [ ] 7. Others (specify)…………………………………………………
9. How often do you buy new seeds? 1. Every season [ ] 2. Yearly [ ] 3. Every two (2) years [ ] 4. Others (Specify)……………………………

10. Which month(s) of the year do you grow tomato?……………………………..

……

11. What tomato variety/varieties do you cultivate and what is/are their yield (s)?

Variety	**Yield (t/ha)**

B. Farmers' knowledge, perception and experiences concerning TYLCV prevalence

12. What are some of the tomato production challenges you encounter in your farm?

Challenges encountered in tomato production		**Tick ( )**
1	Inadequate Finance	
2	Low quality seeds	
3	High cost of fertilizers and insecticides	
4	Pests and Diseases	
5	Lack of Technical Support from Agricultural Extension Agents (AEAs)	
6	Drought	
7	Others (Specify)	

13. What are the major tomato pests you encounter on your farm, their importance and control measures?

Pests	**Level of importance**							**Control Measures**
	1	**2**	**3**	**4**	**5**	**6**	**7**	

1-Most important; 2-Very important; 3-important; 4-Less important; 5-Least important; 6-Not important

14. What are the major tomato diseases you encounter on your farm, their importance and control measures?

Diseases	**Level of importance**							**Control Measures**
	1	**2**	**3**	**4**	**5**	**6**	**7**	

1-Most important; 2-Very important; 3-important; 4-Less important; 5-Least important; 6-Not important

15. Are you aware of the existence of TYLCD? 1. Yes [ ] 2. No [ ]

16. If yes, how did you get to know about it? Through: 1. Personal observation in my farm [ ] 2. Report by other farmers [ ] 3.Report by AEAs [ ] 4. Scientific publication [ ] 5.Others (Specify)…………………………………………………………..

……………………………………………………………………………………………...

17. Do you know the cause of TYLCD? 1. Yes [ ] 2. No [ ]

18. If yes, what cause(s) TYLCV disease? 1. Aphid [ ] 2. Red mite [ ] 3. Mealy bug [ ] 4. Whitefly [ ] 5. Caterpillar [ ] 6. Grasshopper [ ]

7. Others (Specify)……………………………………………………………....................

19. What other crop(s) does the pest that causes TYLCD attack? 1. Pepper [ ] 2. Eggplant [ ] 3. Cassava [ ] 4. Yam [ ] 5. Plantain [ ] 6. Others (Specify)………………………………………………………………………………...

20. What are the symptoms of TYLCV on leaves, stems and fruits of tomato?

Leaves...Stems..

fruits..

21. At what stage of the plant growth do the plants suffer from the disease? 1. At the nursery [ ] 2. 1-2 weeks after transplanting [ ] 3. 3 – 4 weeks after transplanting [ ] 4. 5-6 weeks after transplanting [ ] 5. 7 weeks and above [ ]

22. Which of the tomato varieties you cultivate does the disease commonly affect?

....

23. Which month(s) of the year do you observe TYLCV disease in your farm?

..............................………………………...

24. How often does the disease affect your farm? 1. Every season [ ] 2. Yearly [ ] 3. Every two (2) years [ ] 4. Others(Specify)………………………………………..…….

25. What percentage loss does TYLCV disease have on your yield? 1. 25% yield loss [ ] 2. Between 25% and 50% yield loss [ ] 3. Between 50% and 75% yield loss [ ] 4. Between 75% and 100% yield loss [ ] 5. 100% yield loss [ ]

26. How do you control TYLCV disease? 1. Early planting [ ] 2. Distant old field from new field [ ] 3.Use of mesh screens [ ] 4. Use of resistant cultivars [ ] 5. Practicing crop rotation [ ] 6. Proper farm sanitation [ ] 7.regular weeding [ ] 8. Chemical spraying [ ] 8. Others (Specify)…...................................………………………………………

27. Can the disease be reduced by the use of resistant varieties? 1. Yes [ ] 2. No [ ] 3. No idea [ ]

28. Rank in order of importance the following factors that should be considered in developing a tomato variety for your locality.

Traits	**Level of importance**							**Control Measures**
	1	**2**	**3**	**4**	**5**	**6**	**7**	
High yielding								
Longer shelf life								
Big fruit size								
Drought resistant								

Heat Resistant								
TYLCV resistant varieties								
Others (Specify)								

1-Most important; 2-Very important; 3-important; 4-Less important; 5-Least important; 6-Not important

Table S1: Codes, names and sources of 123 tomato accessions evaluated

Code	Accessions	Local Name	Source	Code	Accessions	Source
G1	GH9116	Tomatose	Eastern Region	G42	14TAT101383	Syngenta
G2	GH9246	Petomec	Eastern Region	G43	14TAT101385	Syngenta
G3	GH9251	RASTER	Eastern Region	G44	14TAT101394	Syngenta
G4	GH9305	Ntose	Eastern Region	G45	14TAT101353	Syngenta
G5	GH9233	Pimplifolium	Eastern Region	G46	14TAT101371	Syngenta
G6	GH9111	REX	Eastern Region	G47	Tomato TO 687 F1 Hybrid	Syngenta
G7	GH 9239	Burkina	Greater Accra	G48	Tapiche	Syngenta
G8	GH 9137	Tomatose	Greater Accra	G49	14TAT101382	Syngenta
G9	GH 9114	Lorry Tyre	Greater Accra	G50	14TAT101342	Syngenta
G10	GH 9281	Tomatose	Volta Region	G51	LA0643	UC DAVIS
G11	GH9152	One Man thousand	Volta Region	G52	08TEP070545	Syngenta
G12	GH 9163	Asante tomato	Western Region	G53	08TEP070546	Syngenta
G13	GH9208	Asante tomato	Western Region	G54	08TEP070547	Syngenta
G14	GH9243	Adwoba	Western Region	G55	08TEP070729	Syngenta
G15	GH9121	Local 1	Western Region	G56	08TEP080187	Syngenta
G16	GH9224	Local 2	Western Region	G57	15TEP070136	Syngenta
G17	GH9098	Fadebegye	Central Region	G58	10TEP080188	Syngenta
G18	GH9190	Ataamba	Central Region	G59	08TEP070549	Syngenta
G19	GH9184	Pimplifolium	Central Region	G60	08TEP070550	Syngenta
G20	GH9073	1R	Northern Region	G61	08TEP070728	Syngenta
G21	GH9185	Local1	Upper East	G62	Tom DIABOU	Wienco
G22	GH9238	Local 4	Upper East	G63	Tom TAMP	Wienco
G23	GH9109	Local 3	Upper East	G64	Tom INDIO	Wienco
G24	GH9285	LOCAL 5	Upper East	G65	Tom 1999	Wienco
G25	GH9128	Local 6	Upper East	G66	Tom 2000	Wienco
G26	GH9158	Local 2	Upper East	G67	Tom3308	Wienco
G27	GH9200	Ashanti 2	Ashanti Region	G68	Tom 4223	Wienco
G28	GH9311	Local 1	Ashanti Region	G69	Tom EMER	Wienco
G29	GH9104	Local 3	Brong Ahafo	G70	Tom MARIA	Wienco
G30	GH9166	IIVT-13	Korea	G71	Tom Sonia	Wienco
G31	GH9131	IIVT-15	Korea	G72	14A112	FOHCREC
G32	GH9107	IIVT-19	Korea	G73	14A113	FOHCREC
G33	GH9160	Bakkeoseu	Korea	G74	Dyvine RZ	Wienco
G34	GH9207	Superdotaerang	Korea	G75	NL	Crop Science
G35	GH9078	Madiso	Korea	G76	Money maker	FOHCREC
G36	GH9150	AVTO 0101	AVRDC	G77	Shaktiman	FOHCREC
G37	GH9247	AVTO 1006	AVRDC	G78	NO7	FOHCREC
G38	GH9235	AVTO 1020	AVRDC	G79	Tropic	FOHCREC
G39	GH9310	AVTO 9802	AVRDC	G80	Strain B	FOHCREC
G40	GH9237	AVTO 9804	AVRDC	G81	Zumorned	FOHCREC
G41	12TAT001641	-	Syngenta			

Table S1: Continuation

Code	Accessions	Source	Code	Accessions	Source
G82	Heinz 1370	FOHCREC	G104	LA0348	UC DAVIS
G83	#15063	FOHCREC	G105	LA3472	UC DAVIS
G84	NS 504	FOHCREC	G106	LA2821	UC DAVIS
G85	NS577	FOHCREC	G107	LA2-225	UC DAVIS
G86	Nirvana	FOHCREC	G108	LA2644	UC DAVIS
G87	Tomaland	FOHCREC	G109	2-175	UC DAVIS
G88	Larisa	FOHCREC	G110	LA4442	UC DAVIS
G89	Nkansah HT	FOHCREC	G111	LA1802	UC DAVIS
G90	Tropimec	FOHCREC	G112	LA4285	UC DAVIS
G91	Boma VF	FOHCREC	G113	LA3044	UC DAVIS
G92	F1 Jaguar +	Technisem	G114	LA2369	UC DAVIS
G93	F1 Thorgal	Technisem	G115	LA3152	UC DAVIS
G94	F1 Nadira	Technisem	G116	2 - 031	UC DAVIS
G95	F1 Cobra 26	Technisem	G117	LA3012	UC DAVIS
G96	Prado F1	Technisem	G118	EMSD 2010 F1	Agrimat
G97	UC 82	Wienco	G119	*Pimpinellifolium* x Roma	BNARI
G98	Roma Tomato	Wienco	G120	BA-4 *Pimpinellifolium* x Wosowoso	BNARI
G99	Tomato Oxheart	Crop Science	G121	BA-5 *Pimpinellifolium x* Wosowoso	BNARI
G100	UN1621E	Crop Science	C-1	GH 9193 (Power Rano)	Bunso
G101	LA3251	UC DAVIS	C-2	Peto Mech	Agrimat
G102	LA2127	UC DAVIS			
G103	LA1793	UC DAVIS			

Table S2: Means of reproductive, yield component and fruit quality traits of 26 of 119 tomato germplasm.

Genotypes	Blocks	NDFFS (days)	TM (days)	RD (days)	Fr/Pl	Weight (g)	Yield (t/ha)	Brix
G1	1	32.23	62.27	29.99	32.39	32.93	39.43	3.80
G102	1	33.23	61.27	27.99	17.7	16.67	20.22	3.70
G21	1	11.23	60.27	48.99	49.5	8.12	20.36	4.50
G23	1	30.23	57.27	26.99	53.29	17.47	36.12	4.10
G37	1	32.23	59.27	26.99	31.94	26.64	38.65	4.60
G52	1	35.23	65.27	29.99	15.06	34.29	31.97	3.90
G67	1	30.23	61.27	30.99	3.54	56.8	33.14	5.00
G68	1	27.23	67.27	39.99	3.12	52.2	29.31	3.80
G70	1	32.23	62.27	29.99	5.97	45.14	30.65	5.40
G72	1	31.23	62.27	30.99	2.45	50.8	28.17	4.60
G74	1	34.23	62.27	27.99	2	56.68	22.07	4.90
G100	2	28.73	57.77	28.99	33.99	25.72	30.67	3.55
G104	2	40.73	65.77	24.99	9.43	41.34	18.8	3.85
G112	2	40.73	100.77	59.99	7.56	39.47	16.3	4.55
G117	2	30.73	100.77	69.99	5.89	20.71	15.78	4.15
G20	2	34.73	61.77	26.99	32.23	34.18	37.99	2.95
G27	2	33.73	57.77	23.99	24.68	32.11	30.72	3.45
G66	2	34.73	65.77	30.99	18.86	61.87	35.37	4.65
G78	2	26.73	58.77	31.99	11.75	49.33	23.13	4.95
G9	2	34.73	61.77	26.99	44.39	30.24	41.33	3.25
G91	2	33.73	61.77	27.99	28.65	50.37	43.12	4.05
G96	2	31.73	61.77	29.99	34.49	43.58	45.23	4.05
G115	3	33.73	55.77	21.99	6.86	51.03	24.53	4.50
G121	3	32.73	52.77	19.99	55.36	9.99	33	5.10
G13	3	27.73	50.77	22.99	140.81	5.6	44.67	5.50
G5	3	24.73	51.77	26.99	215.34	3.25	36.23	5.90
G51	3	28.73	64.77	31.99	2.91	20.93	16.24	4.10
G57	3	51.73	89.77	37.99	4.11	54.32	19.71	3.60
G6	3	31.73	50.77	18.99	58.5	8.18	29.75	5.90
G64	3	35.73	64.77	28.99	14.56	68.25	41.19	4.70
G65	3	33.73	51.77	17.99	16.51	44.38	25.12	4.90
G76	3	30.73	64.77	33.99	15.26	36.77	30.44	4.10
G95	3	29.73	48.77	18.99	18.56	49.66	39.7	2.90
G109	4	24.23	91.77	67.49	5.74	47.42	19.77	5.21
G113	4	27.23	52.77	25.49	7.21	40.26	20.43	4.51
G114	4	29.23	63.77	34.49	10.5	36.64	22.23	3.71
G12	4	22.23	54.77	32.49	24.76	20.07	25.34	5.01
G29	4	22.23	52.77	30.49	75.56	14.99	34.94	5.41
G39	4	28.23	52.77	24.49	175.59	14.22	55.46	5.71
G43	4	21.23	52.77	31.49	15.21	50.25	30.59	3.21
G59	4	27.23	52.77	25.49	24.86	40.37	37.02	4.71
G60	4	27.23	53.77	26.49	14.13	45.54	24.83	5.01

Table S2: Means of reproductive, yield component and fruit quality traits of 26 of 119 tomato germplasm.

Genotypes	**Blocks**	**NDFFS (days)**	**TM (days)**	**RD (days)**	**Fr/Pl**	**Weight (g)**	**Yield (t/ha)**	**Brix**
G73	4	24.23	52.77	28.49	25	47.87	40.21	4.21
G87	4	28.23	52.77	24.49	12.06	50.3	26.49	3.41
G103	5	32.73	93.27	60.49	27.21	17.86	19.63	4.05
G107	5	40.73	68.27	27.49	4.96	50.14	16.09	4.15
G11	5	26.73	54.27	27.49	106.5	15.12	31.67	5.45
G14	5	26.73	54.27	27.49	125.75	16.18	38.12	4.55
G2	5	29.73	54.27	24.49	104.7	18.61	40.41	4.15
G3	5	27.73	55.27	27.49	73.27	22.63	38.5	3.65
G34	5	28.73	55.27	26.49	115.17	19.36	43.65	4.85
G35	5	29.73	54.27	24.49	273.25	13.24	46	5.45
G69	5	28.73	54.27	25.49	26.91	61.15	46.07	2.95
G90	5	34.73	67.27	32.49	12.42	54.3	18.88	4.85
G98	5	31.73	54.27	30	16.59	44.17	24.5	4.15
G120	6	29.23	60.77	31.49	150.64	9.41	46.86	5.1
G22	6	31.23	59.77	28.49	37.84	24.31	37.88	4.9
G31	6	32.23	58.77	26.49	93.74	13.37	34.34	6.2
G41	6	29.23	57.77	28.49	14.86	51.36	34.02	4.6
G49	6	28.23	62.77	34.49	13.07	59.65	34.58	4.2
G71	6	49.23	100.77	51.49	2.91	78.85	21.97	4.4
G8	6	31.23	57.77	26.49	121.64	12.21	49.46	4.9
G80	6	35.23	65.77	30.49	5.02	56.79	22.94	4.2
G81	6	33.23	61.77	28.49	7.16	45.62	23.3	4.7
G82	6	51.23	100.77	49.49	5.84	45.24	17.3	4.8
G83	6	29.23	60.77	31.49	7.69	47.67	25.17	4.3
G105	7	33.73	62.27	28.49	12.61	21.97	21.13	3.95
G118	7	28.73	58.27	29.49	24.77	45.6	37.39	4.15
G16	7	29.73	58.27	28.49	77.27	15.32	35.4	5.65
G24	7	26.73	62.27	35.49	13.93	20.34	21.4	5.75
G25	7	31.73	62.27	30.49	38.66	30.2	36.27	5.25
G32	7	31.73	60.27	28.49	82.83	17.91	39.52	4.95
G45	7	28.73	59.27	30.49	18.19	70.54	38.93	3.75
G54	7	49.73	69.27	19.49	11.58	52.51	25.32	3.95
G61	7	36.73	69.27	32.49	44.14	13.91	25.55	5.85
G75	7	32.73	62.27	29.49	14.57	54.7	32.09	5.05
G85	7	29.73	57.27	27.49	117.72	12.94	35.13	6.45
G28	8	31.23	59.77	28.49	159.82	2.21	45.76	4.91
G44	8	31.23	60.77	29.49	22.98	55.44	32.81	4.61
G46	8	33.23	61.77	28.49	24.53	42.93	31.57	2.91
G47	8	28.23	60.77	32.49	28.16	29.21	28.77	3.81

Table S2: Continuation

Genotypes	Blocks	NDFFS (days)	TM (days)	RD (days)	Fr/Pl	Weight (g)	Yield (t/ha)	Brix
G56	8	67.23	100.77	33.49	16.56	9.04	18.66	4.41
G58	8	32.23	100.77	68.49	55.87	4.14	27.65	5.61
G7	8	17.23	60.77	43.49	71.81	4.61	31.36	6.31
G84	8	29.23	59.77	30.49	30.45	46.23	36.85	3.91
G89	8	29.23	56.77	27.49	29.65	13.61	24.74	4.81
G93	8	28.23	59.77	31.49	52.09	16.16	37.99	4.91
G99	8	33.23	72.77	39.49	20.1	240.43	52.71	4.41
G101	9	32.73	66.77	33.99	1.97	42.93	19.84	2.7
G106	9	28.73	58.77	29.99	21.03	33.28	27.08	4.2
G17	9	27.73	51.77	23.99	66.08	26.03	43.73	4.3
G18	9	24.73	50.77	25.99	65.36	18.53	28.01	5.3
G19	9	27.73	58.77	30.99	88.98	19.67	37.61	2.4
G33	9	29.73	54.77	24.99	66.53	22.57	37.15	4
G36	9	24.73	50.77	25.99	52.08	27.1	39.36	2.4
G53	9	35.73	65.77	29.99	8.51	50.71	28.96	4.6
G62	9	28.73	59.77	30.99	1.63	64.65	28.33	2.5
G94	9	28.73	54.77	25.99	19.13	53.78	44.61	2.3
G97	9	30.73	61.77	30.99	8.36	40.55	22.72	3.4
G108	10	30.73	63.77	32.99	113.21	1.96	25.39	6.45
G110	10	30.73	62.77	31.99	63.89	17.95	42.9	4.85
G116	10	42.73	98.77	55.99	31.43	44.06	21.75	3.65
G26	10	31.73	62.77	30.99	80.21	12.07	39.71	4.95
G30	10	27.73	62.77	34.99	118.26	8.54	47.96	5.15
G38	10	30.73	59.77	28.99	209.13	3.63	53.61	5.05
G40	10	30.73	59.77	28.99	43.1	20.87	34.06	4.75
G42	10	30.73	57.77	26.99	31.48	43.66	36.78	4.35
G48	10	38.73	62.77	23.99	25.42	34.65	26.89	3.85
G55	10	42.73	98.77	55.99	19.01	112.77	19.43	4.55
G63	10	31.73	59.77	27.99	24.57	52.14	29.83	5.15
G10	11	29.73	58.77	28.99	37.77	26.2	36.88	5.1
G111	11	37.73	98.77	60.99	6	23.18	15.15	4.5
G119	11	26.73	59.77	32.99	150.97	13.58	33.44	4.9
G15	11	26.73	59.77	32.99	77.17	19.52	38.34	5.5
G4	11	28.73	59.77	30.99	118.59	14.4	30.13	5.1
G50	11	29.73	58.77	28.99	1.12	52.02	33.11	4
G77	11	25.73	56.77	30.99	1.08	59.07	35.25	4
G79	11	30.73	59.77	28.99	2.37	48.68	30.69	4.9

Table S2: Continuation

Genotypes	**Blocks**	**NDFFS (days)**	**TM (days)**	**RD (days)**	**Fr/Pl**	**Weight (g)**	**Yield (t/ha)**	**Brix**
G86	11	30.73	59.77	28.99	8.28	26.9	22.42	3.9
G88	11	19.73	57.77	37.99	4	73.26	24.03	3.5
G92	11	25.73	58.77	32.99	6	71.77	31.62	3.9
C-1		28.37	56.18	27.76	30.86	30.51	36.79	4.49
C-2		34.1	63.36	29.22	21.58	38.22	32.2	4.01
SED df=10 (checks)		1.24	2.05	1.66	4.18	3.61	5.23	0.24
SED df=10 (within block)		3.05	5.03	4.06	10.23	8.84	12.8	0.59
SED df=10 (different blocks)		4.13	6.81	5.5	13.85	11.97	17.34	0.8

Table S3: List of tomato accessions grouped based on morphological traits

Cluster I		**Cluster II**	**Outlier**
Power Rano	08TEP080187	LA2644	Tomato Oxheart
Peto Mech	15TEP070136	GH9152	
GH9116	08TEP070549	LA4442	
GH 9281	08TEP070550	BA-1 Wild * Roma	
UN1621E	08TEP070728	BA-4 Wild * Wosowoso	
LA3251	Tom DIABOU	BA-5 Wild * Wosowoso	
LA2127	Tom TAMP	GH9208	
LA1793	Tom INDIO	GH9243	
LA0348	Tom 1999	GH9121	
LA3472	Tom 2000	GH9224	
LA2821	Tom3308	GH9098	
LA2-225	Tom 4223	GH9190	
2-175	Tom EMER	GH9184	
LA1802	Tom MARIA	GH9246	
LA4285	Tom Sonia	GH9185	
LA3044	14A112	GH9109	
LA2369	14A113	GH 9158	
LA3152	Dyvine RZ	GH9311	
2 – 031	NL	GH9104	
EMSD 2010 F1	Money maker	GH9251	
GH 9163	Shalctiman	GH9166	
GH9073	NO7	GH9131	
GH9238	Tropic	GH9107	
GH9285	Strain B	GH9160	
GH9128	Zumorned	GH9207	
GH9200	Heinz 1370	GH9078	
GH9247	NS 504	GH9150	
GH9237	Nirvana	GH9235	
12TAT001641	Tomaland	GH9310	
14TAT101383	Larisa	GH9305	
14TAT101385	Nkansah HT	GH9233	
14TAT101394	GH 9114	10TEP080188	
14TAT101353	Tropimec	GH9111	
14TAT101371	Boma VF	GH 9239	
Tomato TO 687 F1 Hybrid	F1 Jaguar +	GH 9137	
Tapiche	F1 Nadira	NS577	
14TAT101382	F1 Cobra 26	F1 Thorgal	
14TAT101342	Prado F1		
08TEP070545	UC 82		
08TEP070546	Roma Tomato		
08TEP070547			

Table S4: Key descriptive statistics for measuring informativeness of 48 of the 338 SNPs markers based on 96 tomato accessions of Ghanaian and Exotic origin

Locus	**N**	**Hobs**	**HExp**	**PIC**	**Locus**	**N**	**Hobs**	**HExp**	**PIC**
S100022	94	0.117	0.502	0.375	S12638	96	0.167	0.481	0.364
S100037	94	0.181	0.502	0.375	S12647	96	0.229	0.489	0.368
S100154	96	0.104	0.492	0.37	S12656	94	0.117	0.291	0.248
S100197	94	0.16	0.277	0.237	S12664	95	0.021	0.081	0.077
S100205	96	0.104	0.492	0.37	S12718	96	0.167	0.44	0.342
S100240	96	0.083	0.355	0.291	S12749	95	0.284	0.438	0.341
S100246	93	0.075	0.093	0.088	S12757	96	0.281	0.436	0.34
S100269	96	0.156	0.349	0.287	S12799	96	0.156	0.392	0.314
S100516	96	0.156	0.496	0.372	S12826	96	0.073	0.162	0.148
S100561	96	0.219	0.479	0.363	S13202	96	0.229	0.447	0.346
S100691	96	0.073	0.436	0.34	S13398	95	0.116	0.43	0.337
S100743	96	0.156	0.5	0.374	S13399	96	0.167	0.44	0.342
S100810	94	0.043	0.174	0.158	S13404	92	0.196	0.499	0.373
S100981	96	0.031	0.179	0.162	S13458	95	0	0.061	0.059
S100987	96	0.115	0.286	0.244	S13481	95	0.263	0.485	0.366
S100995	95	0.168	0.456	0.351	S13625	96	0.042	0.099	0.094
S101009	96	0.052	0.179	0.162	S13762	94	0.16	0.5	0.374
S101067	96	0.083	0.397	0.317	S13842	96	0.219	0.228	0.201
S101068	95	0.084	0.267	0.231	S13868	95	0.074	0.071	0.068
S101085	96	0.115	0.228	0.201	S13899	95	0.105	0.155	0.142
S10372	96	0.135	0.338	0.279	S14323	96	0.104	0.366	0.298
S10686	96	0.125	0.293	0.249	S14354	93	0.14	0.218	0.193
S10796	96	0.073	0.196	0.176	S14355	96	0.135	0.258	0.224
S10958	96	0.125	0.332	0.275	S14415	94	0.255	0.421	0.331
S11092	90	0.133	0.461	0.353	S14458	96	0.188	0.481	0.364
S11205	96	0.146	0.387	0.311	S14530	96	0.198	0.487	0.367
S11231	95	0.2	0.501	0.374	S14653	95	0.2	0.302	0.255
S11281	95	0.063	0.189	0.171	S14758	93	0.172	0.385	0.31
S11543	95	0.211	0.462	0.354	S14868	84	0.774	0.491	0.369
S11588	96	0.063	0.099	0.094	S14890	96	0.125	0.319	0.267
S12200	93	0.161	0.499	0.373	S1490	94	0.191	0.298	0.252
S12201	96	0.198	0.436	0.34	S1498	94	0.16	0.262	0.227
S12212	94	0.117	0.49	0.369	S15013	96	0.052	0.127	0.118
S12213	96	0.115	0.493	0.37	S15039	96	0.156	0.498	0.373
S12372	96	0.156	0.42	0.33	S1504	96	0.167	0.279	0.239
S12414	94	0.138	0.433	0.338	S15046	95	0.105	0.502	0.375
S12421	91	0.11	0.246	0.215	S15058	95	0.137	0.495	0.371
S12501	95	0.053	0.091	0.086	S1525	96	0.198	0.3	0.254
S12535	96	0.063	0.118	0.11	S15432	96	0.24	0.474	0.36
S12536	96	0.063	0.118	0.11	S15515	95	0.179	0.465	0.356

N (Number of individuals typed at the locus), Hobs (Observed heterozygosity), Hexp (Expected heterozygosity), PIC (Polymorphic Information Content)

Table S4: Continuation

Locus	N	Hobs	HExp	PIC	Locus	N	Hobs	HExp	PIC
S1568	96	0.115	0.286	0.244	S18763	96	0.115	0.502	0.375
S15688	95	0.179	0.488	0.368	S18996	91	0.121	0.312	0.262
S15693	92	0.152	0.417	0.329	S19102	96	0.135	0.469	0.357
S1572	95	0.168	0.4	0.319	S19345	96	0.24	0.338	0.279
S15765	95	0.158	0.302	0.255	S19514	92	0.174	0.491	0.369
S15780	96	0.229	0.432	0.337	S19569	96	0.125	0.306	0.258
S15785	92	0.141	0.493	0.37	S19570	96	0.125	0.306	0.258
S15789	94	0.138	0.433	0.338	S19574	96	0.167	0.495	0.371
S15889	96	0.063	0.118	0.11	S19630	93	0.258	0.5	0.374
S16177	95	0.084	0.503	0.375	S19643	96	0.177	0.443	0.344
S16424	96	0.146	0.415	0.328	S19982	95	0.105	0.4	0.319
S16648	96	0.042	0.497	0.372	S19983	96	0.104	0.406	0.323
S16654	95	0.063	0.49	0.369	S20216	96	0.146	0.344	0.283
S16794	96	0.125	0.306	0.258	S20344	92	0.174	0.434	0.339
S16795	96	0.125	0.306	0.258	S20409	96	0.125	0.306	0.258
S16803	96	0.135	0.272	0.234	S20440	89	0.124	0.155	0.142
S16978	96	0.021	0.366	0.298	S21035	94	0	0.27	0.232
S1698	96	0.146	0.495	0.371	S21215	92	0.076	0.382	0.308
S16982	95	0.042	0.487	0.367	S21317	95	0.179	0.395	0.316
S17019	95	0.168	0.503	0.375	S21335	95	0.095	0.328	0.273
S17481	96	0.167	0.501	0.374	S21372	96	0.083	0.293	0.249
S17502	94	0.16	0.5	0.374	S21385	94	0.202	0.49	0.369
S17525	94	0.17	0.488	0.368	S21461	94	0.032	0.355	0.291
S17547	95	0.116	0.476	0.361	S21829	96	0.156	0.498	0.373
S17641	96	0.125	0.502	0.375	S21862	94	0.117	0.502	0.375
S17645	96	0.938	0.503	0.375	S2191	96	0.156	0.5	0.374
S17649	96	0.125	0.502	0.375	S22065	96	0.208	0.502	0.375
S17655	95	0	0.061	0.059	S22109	92	0.293	0.489	0.368
S1772	96	0.208	0.495	0.371	S2234	96	0.146	0.355	0.291
S17756	95	0	0.1	0.095	S22565	95	0.126	0.418	0.329
S17765	96	0.094	0.501	0.374	S22567	95	0.126	0.418	0.329
S17771	96	0.094	0.501	0.374	S22572	93	0.129	0.395	0.316
S1779	95	0.137	0.315	0.264	S22603	95	0.189	0.426	0.334
S18272	91	0.132	0.214	0.19	S22620	96	0.177	0.349	0.287
S18397	96	0.177	0.503	0.375	S22649	95	0.137	0.476	0.361
S18443	96	0.135	0.338	0.279	S22830	95	0.168	0.449	0.347
S18519	96	0.052	0.127	0.118	S231	92	0.022	0.177	0.161
S18619	95	0.189	0.503	0.375	S23192	91	0.132	0.471	0.359
S18641	96	0.156	0.503	0.375	S23480	96	0.188	0.499	0.373
S18739	96	0.115	0.502	0.375	S23589	95	0.168	0.493	0.37

N (Number of individuals typed at the locus), Hobs (Observed heterozygosity), Hexp (Expected heterozygosity), PIC (Polymorphic Information Content)

Table S4: Continuation

Locus	N	Hobs	HExp	PIC	Locus	N	Hobs	HExp	PIC
S23724	92	0.283	0.469	0.357	S34365	95	0.147	0.473	0.36
S23850	95	0.095	0.315	0.264	S34373	96	0.167	0.44	0.342
S24148	96	0.125	0.332	0.275	S34761	96	0.052	0.428	0.335
S24428	90	0.067	0.264	0.228	S3480	93	0.14	0.307	0.259
S24437	94	0.074	0.49	0.369	S36192	91	0.011	0.396	0.316
S24440	95	0.074	0.404	0.321	S36224	93	0.054	0.497	0.372
S24454	96	0.083	0.432	0.337	S36504	96	0.146	0.476	0.362
S24575	96	0.177	0.493	0.37	S36809	95	0.158	0.497	0.372
S24577	94	0.181	0.461	0.354	S37097	94	0.191	0.501	0.374
S25111	93	0.129	0.486	0.367	S37209	93	0.086	0.242	0.212
S2622	95	0.126	0.468	0.357	S37265	93	0.118	0.484	0.365
S2629	96	0.177	0.428	0.335	S39457	92	0.196	0.366	0.298
S26683	95	0.168	0.473	0.36	S39506	88	0.102	0.261	0.226
S2671	90	0	0.393	0.315	S3977	95	0.189	0.468	0.357
S26884	95	0.116	0.302	0.255	S39804	96	0.125	0.489	0.368
S27167	96	0.094	0.228	0.201	S4000	95	0.126	0.295	0.251
S27197	91	0.22	0.428	0.335	S4005	96	0.125	0.293	0.249
S28824	96	0.052	0.313	0.263	S4021	96	0.188	0.492	0.37
S29188	96	0.052	0.212	0.189	S4038	93	0.129	0.3	0.254
S29222	95	0.116	0.414	0.327	S4042	95	0.168	0.462	0.354
S29473	93	0.086	0.454	0.35	S41220	94	0.191	0.298	0.252
S29477	95	0.232	0.502	0.375	S4139	96	0.115	0.463	0.354
S29720	91	0.022	0.471	0.359	S41458	96	0.167	0.344	0.283
S2983	93	0.172	0.477	0.362	S4283	93	0.118	0.499	0.373
S3017	95	0.137	0.352	0.289	S43632	92	0.12	0.477	0.362
S30379	91	0.198	0.503	0.375	S4374	95	0.179	0.495	0.371
S30380	96	0.188	0.503	0.375	S4431	96	0.156	0.502	0.375
S30515	95	0.105	0.334	0.277	S45076	95	0.189	0.295	0.251
S3066	95	0.105	0.449	0.347	S45412	96	0.125	0.489	0.368
S3067	96	0.073	0.402	0.32	S45432	94	0.128	0.488	0.368
S3096	96	0.156	0.498	0.373	S45448	93	0.118	0.488	0.367
S3112	96	0.146	0.415	0.328	S45469	96	0.094	0.286	0.244
S3159	95	0.126	0.4	0.319	S45495	96	0.115	0.493	0.37
S3163	96	0.146	0.454	0.349	S46386	96	0.031	0.313	0.263
S31973	95	0.137	0.315	0.264	S47762	95	0.189	0.478	0.362
S32342	96	0.063	0.355	0.291	S47843	93	0.118	0.333	0.277
S33168	95	0.147	0.334	0.277	S48097	82	0	0.251	0.219
S33701	86	0.151	0.438	0.34	S48121	95	0.084	0.358	0.293
S33745	74	0.095	0.446	0.345	S48426	95	0.021	0.189	0.171
S3430	95	0.147	0.503	0.375	S4926	96	0.156	0.392	0.314

N (Number of individuals typed at the locus), Hobs (Observed heterozygosity), Hexp (Expected heterozygosity), PIC (Polymorphic Information Content)

Table S4: Continuation

Locus	N	Hobs	HExp	PIC	Locus	N	Hobs	HExp	PIC
S4963	93	0.075	0.218	0.193	S62409	96	0.167	0.25	0.218
S5050	91	0.099	0.503	0.375	S62495	96	0.208	0.406	0.323
S50895	96	0.156	0.338	0.279	S6255	96	0.219	0.3	0.254
S50902	96	0.156	0.313	0.263	S62702	95	0.116	0.374	0.303
S50925	96	0.146	0.344	0.283	S6291	96	0.135	0.5	0.374
S50932	95	0.105	0.334	0.277	S63588	96	0.021	0.061	0.059
S5094	96	0.177	0.338	0.279	S63641	96	0.021	0.061	0.059
S5110	94	0.213	0.458	0.352	S63704	96	0.021	0.061	0.059
S51325	95	0.084	0.295	0.251	S63869	94	0.181	0.448	0.346
S51332	93	0.075	0.265	0.229	S65677	94	0.117	0.366	0.298
S51338	96	0.104	0.492	0.37	S6568	96	0.135	0.49	0.369
S51382	96	0.083	0.293	0.249	S65964	96	0.115	0.372	0.301
S51601	96	0.156	0.382	0.308	S6905	93	0.075	0.419	0.33
S5191	96	0.146	0.492	0.37	S69787	96	0.052	0.228	0.201
S5211	95	0.105	0.282	0.241	S69874	94	0.096	0.2	0.179
S53055	93	0.075	0.32	0.268	S69978	96	0.021	0.061	0.059
S53136	95	0.095	0.181	0.164	S7025	94	0.213	0.502	0.375
S55037	93	0.032	0.479	0.363	S7042	93	0.215	0.49	0.368
S5547	96	0.156	0.392	0.314	S7045	95	0.2	0.495	0.371
S5656	96	0.073	0.258	0.224	S706	96	0.146	0.366	0.298
S56956	96	0.083	0.293	0.249	S719	96	0.135	0.361	0.294
S56978	95	0.105	0.493	0.37	S7232	96	0.198	0.5	0.374
S5761	96	0.104	0.25	0.218	S7386	93	0.172	0.482	0.364
S58180	95	0.105	0.418	0.329	S7388	94	0.17	0.429	0.336
S5861	95	0.168	0.462	0.354	S74	94	0.191	0.475	0.361
S5863	91	0.154	0.465	0.355	S7410	94	0.213	0.324	0.271
S58869	96	0.115	0.3	0.254	S75	96	0.177	0.496	0.372
S58916	92	0.098	0.466	0.356	S7775	94	0.128	0.494	0.371
S58945	96	0.188	0.454	0.349	S7829	96	0.146	0.501	0.374
S59159	91	0.088	0.391	0.313	S7919	92	0.098	0.493	0.37
S59771	95	0.147	0.498	0.373	S7939	96	0.094	0.463	0.354
S60078	96	0.094	0.196	0.176	S7942	94	0.085	0.255	0.221
S60360	87	0.057	0.337	0.279	S8064	95	0.105	0.346	0.285
S60417	96	0.063	0.406	0.323	S8223	96	0.063	0.355	0.291
S60557	96	0	0.061	0.059	S8464	96	0.188	0.485	0.366
S60559	96	0	0.061	0.059	S8505	96	0.208	0.502	0.375
S61108	92	0.065	0.442	0.343	S8510	96	0.188	0.387	0.311
S61131	96	0.042	0.415	0.328	S8524	95	0.137	0.289	0.246
S61192	93	0.161	0.475	0.361	S8547	95	0.126	0.267	0.231
S6196	96	0.219	0.228	0.201	S8549	96	0.177	0.361	0.294

N (Number of individuals typed at the locus), Hobs (Observed heterozygosity), Hexp (Expected heterozygosity), PIC (Polymorphic Information Content)

Table S4: Continuation

Locus	N	Hobs	HExp	PIC	Locus	N	Hobs	HExp	PIC
S8669	96	0.125	0.344	0.283	S9379	94	0.043	0.139	0.128
S8697	92	0.13	0.228	0.201	S9444	96	0.156	0.5	0.374
S8774	96	0.156	0.272	0.234	S9510	91	0.473	0.406	0.322
S8787	95	0.084	0.434	0.339	S9663	95	0.116	0.503	0.375
S8807	93	0.075	0.475	0.361	S9681	94	0.138	0.502	0.375
S8835	96	0.198	0.503	0.375	S9689	96	0.031	0.496	0.372
S8855	96	0.094	0.457	0.351	S9703	96	0.104	0.44	0.342
S8858	73	0.055	0.128	0.119	S9707	95	0.158	0.492	0.37
S8859	96	0.052	0.09	0.085	S9856	96	0.25	0.476	0.362

Table S4: Level of heterozygosity of 96 tomato accessions genotyped with 338 SNP markers

Sample	# Loci	# Het loci	Het	Sample	# Loci	# Het loci	Het
GH 9131	337	1	0.003	GH_9243	333	17	0.051
Nkansah HT	335	1	0.003	BA-5xWoso	333	18	0.054
Peto Mech	297	1	0.003	GH 9237	336	19	0.057
Money maker	338	2	0.006	GH 9235	335	19	0.057
LA3472	338	2	0.006	GH 9107	330	21	0.064
GH 9128	337	2	0.006	GH 9160	336	22	0.065
GH_9200	337	2	0.006	GH 9121	333	22	0.066
LA2-225	337	2	0.006	UC_82	333	26	0.078
Tomaland	337	2	0.006	GH 9305	334	27	0.081
LA1793	336	2	0.006	GH 9104	330	27	0.082
LA3012	335	2	0.006	Tropimec	334	28	0.084
Power Rano	335	2	0.006	LA3473	338	30	0.089
Boma VF	334	2	0.006	GH 9238	335	31	0.093
GH 9073	334	2	0.006	GH 9078	336	32	0.095
LA4440B	333	2	0.006	GH 9163	334	32	0.096
UN1621E	332	2	0.006	LA1018	337	34	0.101
Heinz 1370	337	3	0.009	GH_9247	333	34	0.102
LA2821	337	3	0.009	Roma tomato	310	32	0.103
GH 9114	336	3	0.009	BA-1xRoma	324	38	0.117
LA4442	336	3	0.009	GH 9109	333	40	0.12
LA3044	336	3	0.009	GH 9116	313	44	0.141
LA3152	336	3	0.009	GH 9207	331	50	0.151
LA2644	336	3	0.009	#15063	335	63	0.188
LA4440	335	3	0.009	GH 9285	331	65	0.196
GH 9184	335	3	0.009	NS577	330	65	0.197
LA2369	335	3	0.009	GH 9285	331	65	0.196
LA3151	334	3	0.009	NS577	330	65	0.197
LA2127	338	4	0.012	GH 9224	334	71	0.213
LA0348	338	4	0.012	GH_9152	332	71	0.214
GH 9281	334	4	0.012	GH_9098	331	71	0.215

Table S4 Continuation

Sample	**# Loci**	**# Het loci**	**Het**	**Sample**	**# Loci**	**# Het loci**	**Het**
GH 9233	331	4	0.012	Shaktiman	334	76	0.228
GH 9310	334	5	0.015	Nirvana	334	80	0.24
GH 9190	333	5	0.015	Dyvine_RZ	335	81	0.242
GH 9137	333	7	0.021	F1_Nadira	328	89	0.271
Tomato Oxheart	333	9	0.027	Tom_Sonia	333	100	0.3
GH 9239	332	10	0.03	NO7	332	104	0.313
LA1802	336	11	0.033	Zumorned	335	108	0.322
GH 9158	333	14	0.042	F1_Jaguar+	332	108	0.325
GH 9246	334	15	0.045	GH_9150	333	109	0.327
GH 9185	324	16	0.049	Tropic	336	111	0.33
BA-4xWoso	337	17	0.05	Tom_4223	337	112	0.332
GH 9251	308	105	0.341	Tom 2000	337	125	0.371
NS 504	336	116	0.345	NL	336	126	0.375
Tom EMER	337	117	0.347	Tom DIABOU	334	126	0.377
EMSD 2010 F1	334	118	0.353	Tom INDIO	335	130	0.388
GH 9166	333	119	0.357	Tom MARIA	337	131	0.389
14A113	335	123	0.367	Tom 1999	336	132	0.393
Tom 3308	337	124	0.368	Tom TAMP	329	133	0.404
GH 9311	335	124	0.37	F1 Thorgal	332	137	0.413

Loci=Number of loci; #=Number of Heterozygous loci; Het= Heterozygosity